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B. Goldschmidt

RICHARD BENEDICT GOLDSCHMIDT

1878–1958

RICHARD B. GOLDSCHMIDT holds a unique position in the development of the science of Genetics. He worked in the field since its early days and remained an active leader up to his death in 1958. All through this time he has contributed to its growth not only by a vast amount of experimental material but also by the originality of his ideas and speculations which have contributed significantly to the formation and elaboration of the concepts of Genetics.

GOLDSCHMIDT was descended from an old and respected family in Frankfurt am Main. He received the traditional humanistic education, being a member of the last graduation class of the old municipal Gymnasium of Frankfurt, an institution which had a continuous tradition going back to 1524. While the influence of his early classical training appears clearly in GOLDSCHMIDT's scientific work, the stimulus which led him to become a biologist must be found in the activities of the Senckenberg Society which sponsored research in biology and public lectures on various scientific subjects. In this way, it induced many young citizens of Frankfurt, amongst them WEISMANN and BÜTSCHLI, to devote their lives to biology.

GOLDSCHMIDT studied zoology in Heidelberg and Munich, particularly under BÜTSCHLI and RICHARD HERTWIG, and obtained his Ph.D. degree in Heidelberg in 1902. His experiences during this time have become well known through his charming book: "*Portraits from Memory*." He became an Assistant at the Zoological Institute in Munich under RICHARD HERTWIG, and taught there as a Privatdozent and later as Professor extraordinarius until 1913. In 1914, he accepted the direction of a department at the newly founded Kaiser Wilhelm-Institute for Biology, and continued his work there, after 1921 as second Director of the Institute, until 1936. In 1936 he was forced to leave Germany and joined the Department of Zoology at the University of California in Berkeley where he taught until his retirement in 1948 and continued his research and writing until his death.

The first decade of GOLDSCHMIDT's scientific life was devoted to studies in a variety of fields. He published a number of papers on embryological subjects and on the taxonomy of Amphioxus and a series of meticulous studies on the structure and development of the nervous system of Ascaris. His later interest in Genetics is foreshadowed during this early period by a number of cytological investigations dealing with problems of meiosis and germ cell formation, and with the structure and function of the "chromidia" in Protozoan and Metazoan cells. In these studies he developed a picture of the activity of the nucleus in functionally active cells which closely resembles the more recent ideas concerning the relation of DNA and RNA in the nuclear control of cytoplasmic activities.

Since 1910, the major part of GOLDSCHMIDT's scientific work consisted of investigations of different aspects of Genetics. He started out with his classical studies on the geographical variation of the gypsy moth *Lymantria dispar*. These studies which extended over a period of more than 20 years supplied information which had a fundamental impact on two aspects of Genetics, the theory of Evolution and the theory of sex determination.

At the time when GOLDSCHMIDT started his Lymantria work, many geneticists, under the leadership of JOHANNSEN, assumed an agnostic position towards the theory of evolution. GOLDSCHMIDT collected a large number of wild strains of Lymantria from different parts of Europe and from the Far East and subjected them to an exhaustive breeding analysis. He demonstrated that the transmission of the characters distinguishing local varieties, including adaptive characters such as the duration of the diapause, can be analyzed in Mendelian terms and must therefore be regarded as being determined by genes. He established thus the genetic basis of geographical variation long before the theoretical models for gene distributions in populations had been developed by S. WRIGHT, R. A. FISHER and J. B. S. HALDANE.

Even more important was his analysis of sex determination in Lymantria, based on the study of intersexuality occurring in crosses between certain local races. He analyzed his results in genetic terms, establishing a balance between male determining factors carried in the W chromosome and maternally transmitted female determining factors. Both sets of factors may be present in different degrees of "strength" in different local varieties. From this formal genetic analysis, GOLDSCHMIDT proceeded to interpret his results on intersexuality in developmental and physiological terms. In this way, he was the first investigator to bridge the gap between the genetic theory of chromosomal sex determination and the physiological theories of sexual development. Decisive in this respect was his discovery that in Lymantria intersexes there is found a mixture of male and female characters. The regularities he found in this mixture were expressed by GOLDSCHMIDT in the time law of intersexuality: intersexual organs develop up to a certain time in the direction of the sex indicated by the chromosome constitution, then switch over at a particular time of development to the developmental pattern of the opposite sex.

It was characteristic of GOLDSCHMIDT that he did not content himself with this interpretation of his specific experimental results but expanded his conclusions to embrace a much wider range of problems. As he put it himself, in the Introduction of his book "*Theoretical Genetics*": "Though I had the good fortune to contribute a large and diversified mass of factual data in many separate fields of our science, I always made the effort to draw conclusions and extend these to as many groups of facts as I could muster. To satisfy my own need for unified thought, I tried to work generalized, all-embracing ideas into a unified and coördinated system which might be called genetical theory or theoretical genetics." In three books: "*Die quantitativen Grundlagen von Vererbung und Artbildung*" (1920), "*Mechanismus und Physiologie der*

Geschlechtsbestimmung" (1920) and "*Physiologische Theorie der Vererbung*" (1927), the results obtained in Lymantria were expanded into a theory of sex determination, a general theory of gene action, and a hypothesis about the structure of the gene. The "time law of intersexuality" was generalized to mean that genes control biological rates of reaction, both developmental and biochemical. It was suggested, on this basis, for the first time in 1916, that genes control enzymes, a theory which had earlier been proposed by GARROD (1909), HAGEDOORN (1911) and several other authors. The unique feature of GOLDSCHMIDT's hypothesis is the connection of the theory of the enzymatic character of the primary gene products with his generalization of the genic control of developmental reaction velocities based on biochemical reaction rates. On the other hand, the hypothesis is linked up with GOLDSCHMIDT's views on the nature of the genes which are assumed to have themselves enzymatic properties, and are therefore regarded as proteins. He furthermore proposed that different alleles of the same gene differ from each other quantitatively, but not qualitatively.

These theories had an enormous influence on the development of Genetics in the 1920's and 1930's by stimulating both high admiration and violent criticism. Some of his conclusions developed in the 1920's have passed the test of time and constitute now generally accepted fundamental concepts of Genetics, while others, based as they were on the rather primitive biochemical concepts of time, have fallen into oblivion.

In the period following his Lymantria work, GOLDSCHMIDT was next attracted by the developmental aspects of Genetics and the relation between gene and phenotypic character. He chose Drosophila as his experimental object which he continued to use in all his later work. He investigated the interaction of genes and environmental conditions in the production of the phenotype, and studied with great care the phenomenon of "phenocopies," a term which he introduced to designate phenotypic changes resembling gene effects, induced by temperature shocks and other environmental agents. GOLDSCHMIDT interpreted his findings on phenocopies in terms of his theory of developmental reaction rates; his ideas on genic action remained thus fundamentally the same as they had been originally; they became amplified and more concrete but underwent no changes in principle.

On the contrary, GOLDSCHMIDT abandoned his earlier views about gene structure in 1937, under the impact of the establishment of the position effect as a general genetic phenomenon. In his interpretation of the importance of the position effect, GOLDSCHMIDT went much farther than other geneticists of that time since he realized that it made the distinction between gene mutations and chromosomal rearrangements operationally impossible. He abandoned, therefore, the concept of corpuscular genes, ultimate well-defined units of heredity situated in the chromosomes, and substituted for it a pattern of hierarchically arranged series of fields without definite borders inside the chromosomes. These ideas were generally rejected by geneticists at the time but proved to be instrumental in the re-ëvaluation of the gene concept which started in the late 1940's and is still proceeding.

GOLDSCHMIDT's views on the structure of the genic material deeply influenced his thinking on Evolution. Even though he was the first to carry out an extensive genetic analysis of the local variations within a species, and even though he demonstrated, in his Lymantria work, a mechanism leading to sexual isolation between subspecies, he denied, particularly in his book "*The Material Basis of Evolution*," that subspecies, given a mechanism for sexual isolation, would develop into separate species. He assumed, rather, that species formation is a process fundamentally different from race formation, proceeding by means of single mutational events which are qualitatively different from the mutations usually observed and constitute a thorough rearrangement of the genetic material. His studies on the homoeotic mutants of Drosophila, particularly his extensive analysis of the podoptera mutants, which occupy a prominent place in his experimental work in the last 15 years, constitute an attempt to provide an observational basis for his concept of evolution, and offered him an opportunity to integrate his evolutionary and his developmental ideas.

GOLDSCHMIDT did not leave a school in the academic sense. He taught in his early years in Munich, and in his later years in Berkeley, and in both places a number of graduate students worked under him. Although many of his students became famous in their own right, most of them did not continue to elaborate the master's theories but made their work in different fields. During his three trips to Japan, in which he collected much of his Lymantria material and particularly where he served as Professor of Zoology at the University of Tokyo (1924–1926), he exerted a deep influence on the Japanese geneticists and on the development of Genetics in Japan. He also contributed to the teaching of Genetics by writing a textbook at the college level which first appeared as early as 1911 and went through five editions. He carried the knowledge of biology and Genetics into wider circles by writing two books at the popular level, "*Ascaris*," and "*Die Lehre von der Vererbung*" (an English variant of this book appeared under the title "*Understanding Heredity*").

GOLDSCHMIDT's main influence on the development of Genetics was exerted by his writings and by his ideas. They were sometimes received with acclaim, sometimes they suffered violent rejection, but they were never received with indifference. Not infrequently some of his ideas which appeared unorthodox and untenable when first proposed, were later accepted or formed the basis of further elaboration and progress.

GOLDSCHMIDT's contributions were acknowledged by a large number of honors. He held three honorary degrees (Kiel, Madrid, Berlin), was a member of the National Academy of Sciences and a foreign or honorary member in 18 foreign Academies and learned Societies. In 1953, he served as President of the 9th International Congress of Genetics in Bellagio.

During his scientific career of over 50 years, GOLDSCHMIDT produced a vast amount of work, published in over 200 scientific papers and 17 books. He possessed an enormous working capacity, an excellent memory and above all a deep devotion to his scientific work. Even after he had become seriously ill, at

his return from the Bellagio Congress in 1953, he continued his work, writing two books and carrying out several experimental projects. His experimental work is characterized by the large amount of material collected and by the thorough and exhaustive evaluation of the results. He took his own findings, as well as those of other authors, very seriously, and followed the conclusions from the experimental material into all its consequences. In his theoretical work he was aided by his wide knowledge of the literature, both old and modern, and by his original and independent imagination. He never hesitated to publish his speculations, even though his conclusions frequently contradicted commonly accepted ideas. He had a high degree of self-confidence which enabled him to stick to his convictions, even when, in the late 1930's and early 1940's, they met with widespread criticism amongst his colleagues. He was, however, aware of the fact that his views and generalizations represented first approximations to the truth, valid only for the time being, and he did not hesitate to modify or even abandon his ideas in the face of new experimental evidence. It was this willingness to keep up with the rapid progress of Genetics in the last decade and to incorporate the new results into his theoretical thinking which kept him in the forefront of active investigators up to the end.

Through all of GOLDSCHMIDT's work, experimental as well as theoretical, there persists the tendency of unifying the different fields of Genetics into one coherent, integrated picture. He realized already in his early Lymantria work that all the different aspects of Genetics—gene structure, gene action and Evolution—are closely connected with each other and cannot be fully understood in separation from each other. He saw, furthermore, that the findings of Genetics are fundamental for our understanding of the processes of life. In this way, GOLDSCHMIDT's work has been instrumental in putting Genetics into the central position which it occupies at present in the Biological Sciences.

(I want to thank DR. CURT STERN, University of California, Berkeley, California for reading the manuscript and giving me the benefit of his criticisms and suggestions.)

ERNST CASPARI

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1. GENETICS will be changed from a bimonthly to a monthly journal.

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MUTATOR FACTOR IN SALMONELLA TYPHIMURIUM[1]

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SPONTANEOUS mutability is known to be considerably higher in strain LT-7 of *Salmonella typhimurium* than in strain LT-2. Observations, made by many workers in this laboratory, of frequencies of reversion in auxotrophs derived from LT-7 have shown wide variation in rate of reversion among individual mutants. This fact suggested that the laboratory's stock of strain LT-7 is heterogeneous with regard to spontaneous mutability, in other words, that some of the cells have higher mutability than others. In an experiment to test this possibility, the indicator was frequency of mutation from streptomycin sensitivity (*str-s*) to streptomycin resistance (*str-r*). Approximately 100 cells of strain LT-7 were plated on each of three nutrient agar plates, which were incubated overnight at 37°C; 240 colonies picked at random from these plates were resuspended in a small amount of saline and streaked on nutrient agar containing 100 μg/ml of streptomycin. After 20-hour incubation at 37°C, the streaks were observed to be of two types: 90 of them showed many *str-r* mutants, whereas 150 had very few or none. This test confirmed the probability that the LT-7 stock strain is heterogeneous, and indicated that about 40 percent of its cells carry a mutability factor which makes the *str-r* gene more mutable.

In order to find out whether this factor also affects the stability of other genes, 57 auxotrophic mutants were isolated from a strain derived from a colony which in the previous test had shown a high percentage of *str-r* mutation, and 51 auxotrophs from a strain derived from a colony having a low percentage of mutation. In the first group of auxotrophs, 87.7 percent had high reversion frequencies, whereas only 17.6 percent of the second group had high mutability. Thus it appears that the mutability factor effects the stability of a considerable proportion of different genes. To determine whether or not the mutability factor itself is stable, 90 colonies grown from a bacterial culture carrying the factor, and 90 from a culture not carrying it were compared by streaking on nutrient agar that contained streptomycin. All the streaks of the first lot and none of the second lot had a large number of *str-r* mutants.

The evidence of these experiments revealed that a stable mutability factor is responsible for high gene mutability in some cultures isolated from strain LT-7, and also that the strain is heterogenous, about 40 percent of its cells carrying the factor.

[1] Aided by a grant to M. DEMEREC from the American Cancer Society.

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The next step in the analysis was to determine the nature of the factor, that is, whether it is a gene or some cytoplasmic constituent. This investigation was facilitated by the finding that *S. typhimurium* strains carrying the mutator readily hybridize with Hfr strains of *Escherichia coli* K-12. Since a similar mutator is known in *E. coli* (TREFFERS *et al.* 1954) and had been found to be a gene located fairly close to the *thr* (threonine requirement) locus, crosses were made between *S. typhimurium* having the genetic constitution mutator, *thr-61*, *lac*$^-$ (inability to utilize lactose) and *E. coli* HfrCS-101 having the constitution *met* (methionine requirement), *lac*$^+$. Equal volumes of overnight cultures of Salmonella and Escherichia were mixed, and 0.1 ml samples of the mixture were plated immediately on minimal-lactose agar supplemented with threonine (20 μg/ml). After 48-hour incubation at 37°C, *lac*$^+$ recombinants were picked, purified by single-colony isolation, and tested for *thr*$^+$ or *thr* and also for the presence or absence of the mutability factor by streaking on nutrient agar plates containing 100 μg of streptomycin per ml. Out of 65 *lac*$^+$ *thr*$^+$ recombinants, six (or 9.2 percent) did not carry the mutability factor, whereas all of 127 *lac*$^+$ *thr* recombinants did have the factor. Results of experiments with the same parental strains in which *thr*$^+$ recombinants were selected on minimal-glucose agar plates and tested for *lac*$^+$ or *lac*$^-$ and also for the presence or absence of the mutability factor, showed that three out of 61 (4.9 percent) of the *lac*$^+$ *thr*$^+$ recombinants did not carry the factor and four out of 125 (3.2 percent) of *lac*$^-$ *thr*$^+$ did not carry it.

These findings supply good evidence that the mutability factor is a gene, since it recombined in an expected fashion with the genes serving as markers in the crosses. It has been named mutator (*mut*). The results suggest that the order of the three genes tested is *mut—thr—lac*. According to SKAAR (BRYSON *et al.* 1955; SKAAR 1956), *mut* in *E. coli* is located to the right of *thr*; thus, if the two loci are homologous, the gene is located differently in the two species.

Experiments were made with ten alleles of the Salmonella *tryB* locus in order to compare their spontaneous mutability in genomes carrying *mut* and genomes carrying *mut*$^+$. By means of transduction, the *tryB* genes were introduced into the genomes of two *tryD* mutants, one of which (*tryD-7*) is *mut*$^+$ and the other (*tryD-29*) *mut*. Recombinants in which *tryB* replaced *tryD* were selected by plating on enriched minimal agar supplemented with indole, which supports the growth of *tryB* only. Since the *mut* locus is at some distance from the *try* region, it is never included in a *try*-carrying transducing fragment, and thus all recombinants retained the *mut* or *mut*$^+$ marker of the recipient bacteria. Twenty strains obtained in this way, each carrying one of the ten *tryB* genes in combination with either *mut* or *mut*$^+$, were tested for spontaneous mutability. Representative data from one test are given in Table 1. Three plates, each containing about 2×10^9 bacteria, were scored in a test. The results with *tryB-60* and *tryB-62* indicate that these two are stable auxotrophic genes; reversions were not observed on a total of 12 plates each with *mut* and 12 with *mut*$^+$. It seems likely that they originated through chromosomal aberrations. No reversions of *tryB-63* were

TABLE 1

Spontaneous mutability of different tryB *genes in the original genomes carrying* mut$^+$ (*or* mut *in the cases of* tryB-23, -67, *and* -68) *and when transferred to other genomes carrying* mut (*or* mut$^+$)

Allele	Revertants per plate (average three plates) Original	*mut*	*mut*$^+$	*mut/mut*$^+$
tryB-14	4.0	>1000	14.0	>71
-16	2.7	1147	8	143
-17	5.7	75	1.3	58
-18	0.3	1.7	0.3	6
-60	0	0	0	...
-62	0	0	0	...
-63	0	32	0	...
-23	155	148	0.3	493
-67	37	31	4	8
-68	1620	1620	6	270

observed on eight plates among the bacteria carrying *mut*$^+$, but 155 revertants were found on eight plates with *tryB-63 mut* cells.

In all seven of the *tryB* alleles that underwent spontaneous mutations when *mut*$^+$ was present, frequency of mutation was considerably increased by the presence of *mut*. The increase was not uniform, being six-fold in *tryB-18* and about 500-fold in *tryB-23*. The relative degree of increase in frequency does not depend on the relative frequency in the presence of *mut*$^+$, as is especially evident in the data for *tryB-23* and *tryB-67*. The action of the *mut* gene closely resembles that of various mutagenic agents (DEMEREC 1953) in that it affects the stability of different genes of a genome in a highly specific way.

SUMMARY

A mutator gene (*mut*) in *Salmonella typhimurium* affects the stability of all other markers of the genome with which it has been tested. The mutant form of the gene increases spontaneous mutability to different degrees in different alleles of the same locus; in this specificity of effect the action of *mut* is like that of various mutagenic agents. The high mutability observed in strain LT-7 is due to the presence of *mut* in about 40 percent of the cells of a population. The mutator gene is located to the left of *thr-leu* on the Salmonella chromosome.

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DOUBLE CROSSING OVER OR GENE CONVERSION AT THE WHITE LOCI IN DROSOPHILA MELANOGASTER?

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OPERATIONALLY speaking, gene conversion describes those instances of reversion to wild type which are recovered almost exclusively among the progeny of individuals heterozygous for two alleles or pseudoalleles of independent origin. Conversion may be distinguished from double crossing over within a short genetic interval by the apparent absence of the expected reciprocal crossover class. In addition conversion differs from reverse mutation by the significantly lower reversion frequency of the "convertible" mutants when tested in the homozygous state.

Whereas gene conversion appears to be of widespread occurrence in "lower" organisms, e.g. Neurospora and Saccharomyces—and may, in fact, represent recombination in bacteriophage—it appears, with the exception of the reversions of the mutant reddish in *Drosophila virilis* (DEMEREC 1928), to be comparatively infrequent in "higher" organisms. Recently, reversions to wild type among garnet (*g*) eye color pseudoalleles in *D. melanogaster* have been attributed to conversion (CHOVNICK 1958; HEXTER 1958). Since the occurrence of the *g* reversions cannot be unambiguously distinguished from double crossing over, it is relevant to report here a comparable situation among mutants of the white (*w*) eye color pseudoalleles where a distinction can be more readily made.

The results of crosses involving *w* pseudoalleles w^{a2}, w^{bf}, w^{ch}, and w^{col} are of interest here. As crossover markers to the left, the recessive mutants *y* or y^2 (= yellow body), *ac* (= achaete bristles) and *sc* (= scute bristles) were used; *spl* (= split bristles and eye) served as the right side marker.

Parental females were in all cases made heterozygous for the autosomal inversions *Cy* (= Curly wings) and Ubx^{130} (= Ultrabithorax halteres) and were crossed without exception to *y w spl* sn^3 males.

Included in Table 1 are the results of three crosses (1–3) involving the several *w* pseudoalleles where two types of recombinant exceptions were recovered. The exceptional progeny listed in the two left-hand columns occurred in all cases in association with exchange of markers. These exceptions are identical in all respects to exceptions occurring in comparable crosses where the mutant w^a replaced w^{a2} (GREEN 1959) and can, therefore, be explained as the consequence of single crossovers occurring between pseudoallelic mutants. The white-eyed exceptions represent the coupled double mutant crossover products complementary to the wild type crossovers.

Those exceptions listed in the right-hand columns occurred in the absence of marker exchange and therefore arose either without crossing over or with double crossing over. Among the latter five are reversions of w^{a2} and one of w^{col}.

TABLE 1

Exceptional progeny derived from females of several genotypes
(See text for details)

Genotype parental females	Phenotype and number of exceptional progeny				Total progeny
1. *y ac* w^{col} *spl*/w^{a2}	y ac w / 5	w^+ spl / 4	w^+ / 3	y ac w^+ spl / 1	362,116
2. *sc* w^{bf} *spl*/w^{a2}	sc w / 2	w^+ spl / 2	w^+ / 1		93,492
3. *y ac* w^{ch} *spl*/y^2 w^{a2}	y^2 w spl / 10	y ac w^+ / 8	y ac w spl / 1	y^2 w^+ / 1	142,390
4. y^2 *su-*w^a w^a *spl*/w^{a2}			$w^+ = 1$		463,052
5. y^2 *su-*w^a w^{a4} *spl*/w^{a2}			$w^+ = 1$		100,015
6. y^2 *su-*w^a w^{aM} *spl*/w^{a2}			$w^+ = 1$		83,412
7. y^2 *sc* w^{a57a} *ec*/w^{a2}			$w^+ = 1$		111,552

Spontaneous back mutation was first tested as a possible mode of origin of the presumed noncrossover reversions to wild type. Reversions were sought among the progeny of homozygous w^{a2} females. None were found among a total of 459,237 w^{a2} chromosomes scored. This finding suggested that spontaneous reverse mutation does not satisfactorily explain the occurrence of w^{a2} reversions and leaves the question of "conversion" open.

The possibility was next entertained that the *y ac w spl* exception found among the progeny of w^{ch}/w^{a2} females (cross 3) might provide a clue to the origin of the noncrossover exceptions. What suggested itself was that the *y ac w spl* and y^2 w^+ exceptions are complementary to one another. This would follow if double crossing over took place such that one exchange occurred between the pseudoalleles and the other between w^{a2} and the *y ac* loci. Accordingly, the *y ac w spl* exception should carry both w^{a2} and w^{ch} coupled to the same X chromosome. To test this possibility, females of the genotype *y ac w spl*/+; *Cy*/+; *Ubx*/+ were obtained, crossed to *y w spl* sn^3 males and recombinants sought. Among 98,060 progeny, three exceptions were found: two males w^{ch} *spl* and one female w^{a2}/*y w spl* sn^3. These exceptions conclusively establish the genotype of the *y ac w spl* exception as carrying both w^{a2} and w^{ch} and support the double crossover origin of it and its apparent complementary w^+ exception. Of added significance here is the fact that the w^{a2} extracted from the *y ac w spl* chromosome apparently occurred as a similar double crossover. Those w^+ exceptions without markers derived from females w^{a2}/w^{col} and w^{a2}/w^{bf} can be collectively explained by the occurrence of a double crossover event in which one exchange occurred between w^{a2} and w^{col} or w^{bf} and the second between w^{a2} and *spl*. Alternatively the *y ac* w^+ *spl* exception requires a double crossover where one exchange occurred between w^{a2} and w^{col} and the second between w^{col} and *y ac*. Additional support for this interpretation came from the following experiments. The *y ac w* exceptions in cross 1

should be of the genotype *y ac* w^{col} w^{a2}. If double crossing over occurs, it should be possible to extract either w^{col} or w^{a2} from this chromosome. Therefore, females of the genotype *y ac* w^{col} w^{a2} *spl*/+; *Cy*/+; *Ubx*/+ were synthesized and crossed to *y w spl* sn^3 males. Among 233,780 progeny the following three exceptions were found: one male w^{ch} *spl*, *Cy*/+; one female w^{ch} *spl*/*y w spl* sn^3 and one male w^{a2}, *Ubx*/+. The latter presumably arose by a double crossover event identical to that which gave rise to the w^+ exceptions described above. In a comparable experiment where females *y ac* w^{a2} w^{ch} *spl*/+; *Cy*/+; *Ubx*/+ were crossed to *y w spl* sn^3 males, a total of 133,655 progeny were scored. The following exceptions all explainable by the occurrence of single crossovers between w^{a2} and w^{ch} were found: five *y ac* w^{a2} and seven w^{ch} *spl*. No double crossovers were found.

Also listed in Table 1 are four w^+ exceptions which came from females heterozygous for w^{a2} and either w^a (or its partial back mutants w^{aM} and w^{a52a}) or w^{a4}. In these and extensive other crosses among these mutants no other w^+ exceptions were found, nor were any other exceptions which could be explained by means of crossing over between pseudoallelic mutants. In so far as can be concluded from the recombination data, these mutants disjoin as genuine alleles.

The two-fold dilemma posed by the latter results can be succinctly stated as follows. Is there a mechanism of reversion common to all the "noncrossovers" wild types listed in Table 1 or are two or more mechanisms involved? Why have all the reversions been recovered exclusively from females heterozygous for w^{a2} and have involved primarily the w^{a2} chromosome?

While the occurrence of double crossing over in a short interval appears to adequately explain the "noncrossover" exceptions from crosses 1–3, it is inadequate for the others (crosses 4–7) unless it is assumed that w^{a2} and w^a are pseudoalleles and the frequency of double crossing over exceeds the frequency of single crossing over between them. Precedence for the occurrence of double crossovers in a short interval can be found in the studies of pseudoallelism in Aspergillus (PRITCHARD 1955), but as yet no convincing case has been described where the double crossovers exceed the singles for a particular interval. Perhaps all the difficulties encountered stem from the nature of the mutant w^{a2} itself. The fact that all the exceptions involve this mutant suggest that when the genetic properties of w^{a2} are more adequately understood, the mode of origin of the "noncrossover" exceptions will follow. For the time being neither double crossing over nor conversion (whatever the latter connotes) fully explain the aforementioned results.

SUMMARY

Comparatively infrequent reversions to wild type of the mutant w^{a2} of *D. melanogaster* are recorded. Genetic evidence is submitted to show that some of the reversions can be accounted for by rare double crossovers; other reversions cannot be so explained. The implications of these observations are fully discussed.

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STIPPLED ALEURONE IN MAIZE[1]

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THE *R* locus in maize is situated on the long arm of chromosome 10, the shortest chromosome of the complement. The alleles at this locus, when present with certain other complementary genes, condition anthocyanin formation in the aleurone cells of the endosperm and in certain vegetative structures. The four common *R* alleles were designated by EMERSON *et al.* (1935) as follows:

R^r—self-colored aleurone, colored plant
r^r—colorless aleurone, colored plant
R^g—self-colored aleurone, green plant
r^g—colorless aleurone, green plant

Three aleurone "pattern" alleles also occur: stippled (R^{st}), marbled (R^{mb}), and Navajo (R^{nj}). Stippled and marbled give characteristic deeply colored spots in the otherwise colorless aleurone, and are associated with green plant color; the pigment in Navajo kernels is typically restricted to the crown region. Only casual mention of the pattern alleles had been made in the literature (EMERSON *et al.* 1935; STADLER 1942, 1954) until recently when BRINK (1956) and BRINK and WEYERS (1957) described an unusual effect of R^{st} and R^{mb} on R^r action among the offspring of R^rR^{st} and R^rR^{mb} heterozygotes. The effect is one of impairment of the pigmenting capacity of the R^r gene, and is readily observed in the R^rrr endosperms resulting from the pollination of *rr* silks with R^rR^{st} or R^rR^{mb} pollen. It has been shown (BRINK and MIKULA 1957) that R^{st} and R^{mb} also affect the plant-pigmenting action of the R^r gene. The change thus induced in R^r is heritable, although partial reversion toward normal R^r expression regularly occurs. The process by which R^r is changed in R^rR^{st} and R^rR^{mb} heterozygotes has been termed "paramutation" by BRINK (1958).

The previously unsuspected paramutagenic action of R^{st} in R^rR^{st} plants prompted the present study of the R^{st} allele. The investigation is concerned mainly with (1) the relation between the stippled and light stippled phenotypes, which was found to involve a modifier linked with the *R* locus, and (2) mutability of the R^{st} allele.

MATERIALS AND METHODS

Stocks used in these experiments, with certain exceptions noted later, were sublines of a uniform Wisconsin dent inbred strain, W22. These sublines were

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prepared by crossing the appropriate genotypes recurrently to W22 for three or more generations, and then selfing to obtain the desired homozygotes. The constitutions of the sublines thus developed, at loci relevant to this study, were as follows: A_1CR^r, A_1CR^{st}, A_1Cr^r, and A_1Cr^g.

A preliminary examination of a small population of R^rR^{st} ears that had been selfed disclosed occasional stippled kernels with a much reduced frequency of colored spots. Several such light stippled kernels were planted and produced ears that, when selfed, continued to show the light stippled phenotype.

Both the R^{st} and light stippled (R^{st}(light)) phenotypes consist of colored spots on a colorless background. The lighter expression of R^{st}(light) is due to a decrease in frequency, rather than in size, of spots; the intensity of pigmentation in the colored areas is the same. R^{st} and R^{st}(light) also show dosage effects in combinations with r. Figure 1 illustrates R^{st} and R^{st}(light) kernels when homozygous, and when carrying two doses of r^r in the triploid endosperm.

A comparison was made of the frequency of colored spots per unit area on $R^{st}r^rr^r$ and R^{st}(light)$/r^r/r^r$ kernels. The aleurone spots were counted in an area

FIGURE 1.—Dosage effects of R^{st} and R^{st}(light) with r^r. The endosperm genotypes are, from top to bottom: row 1—$R^{st}R^{st}R^{st}$, row 2—R^{st}(light)$/R^{st}$(light)$/R^{st}$(light), row 3—$R^{st}r^rr^r$, row 4—R^{st}(light)$/r^r/r^r$.

of about 5 mm², under a binocular microscope fitted with a 10 × 10 reticule, at 30 × magnification. The reticule was positioned on the abgerminal face of the kernel by centering it across the width of the kernel, setting the top line of the reticule at the upper edge of the kernel, and then moving the scale down five spaces. Two hundred kernels of each genotype were scored, comprising 50-kernel samples from each of four ears. The average number of spots per kernel on $R^{st}r^{r}r^{r}$ endosperms was 20.3, with a range of 9–40; the average number of spots per kernel on R^{st}(light)$/r^{r}/r^{r}$ endosperms was 1.8, with a range of 0–17. Thus, the frequency of spots on $R^{st}r^{r}r^{r}$ endosperms was approximately 11 times greater than on R^{st}(light)$/r^{r}/r^{r}$ endosperms.

The paramutagenic action of R^{st}(light) was tested in heterozygotes with R^{r} and found to be similiar to that of R^{st}, however, a critical comparison of the paramutagenic action of R^{st} and R^{st}(light) was not made.

R^{st}(light) was thought at first to be a mutant form of R^{st}, and tests were made to determine its frequency of occurrence on this assumption.

A series of crosses was made to measure the frequency of changes of R^{st} to R^{st}(light), to self-color (R^{sc}), and to colorless (r). The matings made, and the nonparental phenotypes selected were as follows:

Crosses		Nonparental phenotypes selected
$R^{st}R^{st}$	× $r^{g}r^{g}$	R^{sc}, R^{st}(light), r
$R^{r}R^{st}$		R^{st}(light), r
$R^{st}r^{r}$		R^{sc}, R^{st}(light)

The stability of R^{st}(light) was similarly tested as follows:

Crosses		Nonparental phenotypes selected
R^{st}(light)$/R^{st}$(light)	× $r^{g}r^{g}$	R^{sc}, R^{st}, r
R^{r}/R^{st}(light)		R^{st}, r
R^{st}(light)$/r^{r}$		R^{sc}, R^{st}

Since R^{st} and R^{st}(light) give green plant color, and R^{r} and r^{r} give red plant color, the heterozygous combinations tested carried a red and a green plant color allele. This fact made it possible to identify the source of the nonparental phenotypes listed at the right, above. Exceptional offspring with red plant color were presumed to carry a mutation involving the parent allele with red plant color— R^{r} or r^{r}, exceptional offspring with green plant color were presumed to carry a mutation involving the parent allele with green plant color— R^{st} or R^{st}(light). These assumptions are valid in so far as intrinsic mutations at the R locus are concerned. STADLER and his associates, however, have presented evidence that the seed and plant color functional components of the R locus are separable by crossing over, and that in certain chromosome pairing configurations crossovers are possible that give rise to phenotypes indistinguishable from mutations (STADLER 1951a, 1951b; STADLER and NUFFER 1953; STADLER and EMMERLING 1954, 1956). The assumptions made above do not take into consideration this

possible source of mutant phenotypes. It was possible, however, to test certain mutants for their association with crossing over either distal or proximal to *R* (see DISCUSSION).

Mutation rates were measured in the female only, so that contaminants arising from extraneous pollen could be positively excluded. The total number of kernels on each ear was determined at the same time as the presumed mutants were selected. Ears segregating R^{st} or R^{st}(light) and R^r or r^r were shelled, and the number of kernels was counted for each class. Kernels on homozygous ears were counted on the ear, using a wax pencil to mark the kernels as they were counted.

The presumed mutant kernels were germinated in the greenhouse, and the seedlings were then transplanted to the field. The ears on these plants were pollinated with $R^r r^g$ to verify the mutants and to identify the individuals resulting from pollen contamination. Bona fide mutants segregated the mutant phenotype and r^g, whereas exceptional phenotypes due to pollen contamination did not segregate r^g. The use of $R^r r^g$ pollen served also to provide R^r/mutant heterozygotes, so that a test of the paramutagenic action of the mutants could be made.

Rates of mutation are reported as the number of mutations per 10^4 gametes tested. The loss of presumed mutants, due to failure to germinate or death of the plant before pollination, was compensated for in the computations by proportionately adjusting the total number of kernels.

RESULTS

Alteration of stippled expression

The results of the tests on the frequency of occurrence of R^{st}(light) kernels following pollination of $R^{st}R^{st}$, R^rR^{st} and $R^{st}r^r$ plants with $r^g r^g$ are shown in Table 1. Only the light stippled kernels that transmitted this phenotype are entered in the tabulation.

The extreme difference in the frequency of R^{st}(light) kernels on the homozygous and the two classes of heterozygous ears suggested two possible explanations: (1) R^{st}(light) arises as a mutation from R^{st}, and the rate of mutation is greatly increased by heterozygosity with R^r or r^r, or (2) R^{st}(light) is not a mutation from R^{st} but is a product of crossing over between R^{st} and a linked modifier carried on

TABLE 1

Frequency of occurrence of R^{st} *(light) kernels on* $R^{st}R^{st}$, R^rR^{st}, *and* $R^{st}r^r$ *ears pollinated with* r^gr^g

Genotype	Number of kernels		No. of R^{st}(light) kernels per 10^4 R^{st} gametes tested
	R^{st}	R^{st}(light)	
$R^{st}R^{st}$	18,586	5*	2.7
R^rR^{st}	11,028	688	587.2
$R^{st}r^r$	2,056	109	503.9

* Two of these mutations were somatic and produced R^{st}(light) ear sectors; one sector was comprised of nine kernels and the other of three kernels. Each was counted as one mutation.

the R^r and r^r chromosomes. Additional experiments were conducted to test these two alternatives.

The first test utilized markers proximal and distal to the *R* locus to determine whether R^{st} (light) kernels on R^gR^{st} ears were associated with crossing over. The two markers used were golden (*g*), 14 crossover units proximal to the *R* locus, and a large heterochromatic knob (*K*), distal to *R* that shows approximately one percent recombination with the latter. Golden plants are yellow-green in color at the flowering stage, and *K* causes preferential segregation in megasporogenesis in favor of the *K* chromosome (RHOADES 1942, 1952). The following cross was made:

$$g\ R^g\ K\ /\ G\ R^{st}\ k \times g\ r\ k$$

The stocks used in this mating were not sublines of W22. R^{st} (light) kernels were selected from the ears from the cross. The kernels were planted, and the resulting plants were scored for golden. The ears produced by the plants were pollinated with *rr*. One hundred kernels were shelled from each of these ears, and counts were made of the number of R^{st} (light) and *r* kernels. The ratios of R^{st} (light):*r* kernels ranged from 65:35 to 87:13, results that would be expected if the R^{st} (light) chromosome carried *K*. As a control, the plants and ears from 116 R^{st} kernels were scored for golden and *K*, respectively. Scoring for *K* on these ears was done by visual examination of the kernel populations, rather than by actual kernel count.

The results from the scorings are presented in Table 2. They show that the occurrence of R^{st} (light) kernels was always associated with crossing over between R^{st} and *K*, the distal marker.

The data presented in Table 2 indicate that the R^{st} (light) kernels from the heterozygous combinations in Table 1 are the result of crossing over between R^{st} and a distal modifier, and are not mutations from R^{st}. The modifier that conditions the R^{st} phenotype will be designated M^{st}, and the one that conditions the R^{st} (light) phenotype, m^{st}. In the test referred to in Table 2, the R^{st} chromosome, therefore, would be designated $G\ R^{st}\ M^{st}k$ and the R^g chromosome, $g\ R^g\ m^{st}K$. Both the R^r and r^r chromosomes in the heterozygous combinations in Table 1 must have carried m^{st}. In these terms, an R^{st} (light) chromosome resulting from a crossover would be of the constitution, $R^{st}\ m^{st}$.

If the number of R^{st} (light) kernels from the two heterozygous combinations referred to in Table 1 are considered a crossover class, and the R^{st} kernels a

TABLE 2

Distribution of the markers g *and* K *in* R^{st} *and* R^{st} (*light*) *kernels from the cross:* g R^g K / G R^{st} k × g r k

Genotype	Noncrossover		Crossover		Total
	G k	*g k*	*G K*	*g k*	
$R^{st}r$	105	11	0	0	116
R^{st} (light)/*r*	0	0	39	0	39

parental class, the frequency of crossing over between R^{st} and the modifier can be calculated as approximately 5.9 percent in the R^rR^{st} heterozygotes, and 5.0 percent in the $R^{st}r^r$ heterozygotes. The pooled data from the two heterozygous combinations give a crossover frequency of 5.7 percent.

Mutations of R^{st} and R^{st}(light) to R^{sc} were used in a further test of the hypothesis that the light stippled phenotype differs from stippled in a linked modifier. It was assumed that a mutation of R^{st} or R^{st}(light) to R^{sc} was a mutation at the R locus, and that the linked modifier remained unchanged. Self-colored mutants from R^{st} and R^{st}(light) were made heterozygous with R^{st}, and the ears produced by these individuals were pollinated with r^gr^g. The ears from the two groups of matings were then scored for the presence or absence of R^{st}(light) kernels. The results are shown in Table 3. The data presented in Table 3 are as expected if R^{st}(light) kernels result from the action of a linked modifier, and mutations of R^{st} or R^{st}(light) to R^{sc} do not alter the action of the modifier. The occurrence of R^{st}(light) kernels on nine ears from the R^{sc} R^{st} heterozygotes when R^{sc} is a mutation from R^{st} was to be expected, because R^{sc} was isolated originally from a heterozygote that, by hypothesis, carried an r^r m^{st} chromosome. Thus, m^{st} could have been acquired by crossing over. The observed frequency of such exceptional ears approximates the expected value of 5.7 percent.

An additional test of the linked modifier hypothesis was based on the prediction that R^r and r^r chromosomes carrying M^{st}, rather than m^{st}, would be recoverable from appropriate heterozygotes. Such R^r and r^r chromosomes, in heterozygotes with R^{st}, should not produce R^{st}(light) offspring as crossovers. Two tests were made to verify this prediction:

(1) From R^rR^{st} heterozygotes 5.7 percent of the R^r chromosomes should carry M^{st} rather than m^{st}, and when the R^r on this chromosome is made heterozygous with R^{st}, R^{st}(light) offspring should not arise as a result of crossing over. R^rR^{st} plants were selfed, and the colored seeds from these ears (R^rR^r and R^rR^{st}) were planted. The resulting ears were pollinated with r^gr^g. The homozygous R^r ears were discarded. One hundred and seventy-eight R^rR^{st} ears were scored for the presence or absence of any R^{st}(light) kernels. Nine, or 5.1 percent, were found that were free of R^{st}(light) kernels. This frequency was tested by χ^2 against an expected value of 5.7 percent, and difference was found to be not significant.

(2) The following cross was made to obtain an r^r chromosome that carried M^{st}, rather than m^{st}:

$$R^{st}r^r \times r^gr^g$$

TABLE 3

Occurrence of R^{st}*(light) kernels on* $R^{sc}R^{st}$ *ears pollinated with* r^gr^g, *when* R^{sc} *is a mutant from* R^{st} *and* R^{st}*(light)*

Genotype	Source of R^{sc} mutant	Number of ears R^{st}(light) kernels Present	Absent	Total
$R^{sc}R^{st}$	R^{st}	9	96	105
$R^{sc}R^{st}$	R^{st}(light)	31	0	31

The colorless kernels from the cross ($r^r r^g$) were planted, and the ears were pollinated with homozygous R^{st}(light). If the hypothesis in question is valid, approximately 5.7 percent of the r^r chromosomes should carry M^{st} as a result of crossing over in the $R^{st} r^r$ parent. It was anticipated that R^{st}(light) would have a darker phenotypic expression when heterozygous with $r^r M^{st}$ than when heterozygous with $r^g m^{st}$. The results expected from this test may be diagrammed as follows:

	Endosperm genotypes
Noncrossovers:	
$r^r m^{st}/r^g m^{st} \times R^{st}$(light)$/R^{st}$(light)	(1) $r^r m^{st}/r^r m^{st}/R^{st} m^{st}$
	(2) $r^g m^{st}/r^g m^{st}/R^{st} m^{st}$
Crossovers:	
$r^r M^{st}/r^g m^{st} \times R^{st}$(light)$/R^{st}$(light)	(1) $r^r M^{st}/r^r M^{st}/R^{st} m^{st}$
	(2) $r^g m^{st}/r^g m^{st}/R^{st} m^{st}$

The two classes of kernels on the ears in the noncrossover group should be phenotypically indistinguishable. In the crossover group, however, the class of kernels that carries two doses of M^{st} and one of m^{st} should have a darker phenotype than the class that carries three doses of m^{st}. The ears were inspected to determine whether any showed two different stippled phenotypes in a ratio of 1:1. Only 31 ears were obtained in the test, but two of them segregated dark and light stippled kernels. The ratio of dark:light kernels on these two ears was 140:131 and 61:74. If the dark stippled phenotype is associated with the modifier on the r^r chromosome these kernels should give red seedlings when germinated; if the light stippled phenotype is associated with the modifier on the r^g chromosome these kernels should give green seedlings when germinated. Kernels from the light and dark classes from each ear were germinated and scored for seedling color, with the following result:

	No. of seedlings	
	red	green
Light class	8	131
Dark class	123	12

The few red seedlings in the light class and green seedlings in the dark class were expected as crossovers. The observed frequency of these crossovers was tested by χ^2 against an expected value of 5.7 percent, and the difference was found to be not significant.

Mutability of stippled

The results of the tests on the frequency of mutation of R^{st}(light) to R^{st} are shown in Table 4. Two hundred and seventy-two kernels were selected from the three crosses referred to in Table 4 as possible mutations to R^{st}. Thirty-one of these kernels failed to produce a plant, and of the 241 that did produce plants none were mutations to R^{st}. The 241 nonmutants were of the following constitutions: 234 R^{st}(light), one R^{sc}, and six rogues resulting from pollen contamination.

TABLE 4

Frequency of mutation of R^{st} *(light) to* R^{st} *when homozygous, and when heterozygous with* R^r *and* r^r

Genotype	Number of kernels R^{st} (light)	Number of kernels R^{st}	No. of R^{st} mutation per 10^4 gametes tested
R^{st} (light)/R^{st} (light)	24,367	0	0
R^r/R^{st} (light)	4,675	0	0
R^{st} (light)/r^r	5,172	0	0

TABLE 5

Frequency of mutation of R^{st} *and* R^{st} *(light) to* R^{sc} *in homozygotes, and in heterozygotes with* r^r

Genotype	Number of kernels R^{st} or R^{st} (light)	R^{sc}	No. of R^{sc} mutations per 10^4 gametes tested
$R^{st}R^{st}$	19,920	34	17.0
R^{st} (light)/R^{st} (light)	24,599	49	19.9
$R^{st}r^r$	2,055	1	4.9
R^{st} (light)/r^r	4,623	2	4.3

TABLE 6

Frequency of mutation of R^{st} *and* R^{st} *(light) to colorless or near-colorless in homozygotes, and in heterozygotes with* R^r

Genotype	Number of kernels R^{st} or R^{st} (light)	Number of kernels Colorless and near-colorless (green plant color)	No. of colorless and near-colorless mutations (green plant color) per 10^4 gametes tested
$R^{st}R^{st}$	20,825	0	0
R^{st} (light)/R^{st} (light)	26,805	1	0.4
R^rR^{st}	10,942	6	5.4
R^r/R^{st} (light)	4,720	2	4.2

The results of the tests on the frequency of mutation of R^{st}, and R^{st} (light) to R^{sc} are shown in Table 5. The R^{sc} mutation rate data from the R^{st} and R^{st} (light) homozygotes were pooled and compared, by a χ^2 test of independence of assortment, with the pooled R^{sc} mutation rate data from the R^{st} and R^{st} (light) heterozygotes with r^r. The difference between the rate of mutation in the homozygotes and heterozygotes was found to be highly significant.

It has recently been shown (KERMICLE, personal communication) that a few stippled kernels from the cross $R^{st}\ r^g \times r^g\ r^g$ have colored scutellum, and that when such kernels are planted to determine their germinal constitution many prove to be germinally R^{sc}. The cause of the noncorrespondence between germ and endosperm is not known. The rates of mutation to R^{sc} reported in this paper do not include this exceptional class of mutants, but the data lose none of their value for comparative purposes.

The results of the tests on the frequency of mutation of R^{st} and R^{st} (light) to colorless or near-colorless are shown in Table 6. Plants grown from the kernels

selected as colorless mutants from the heterozygous ears were observed for plant color, and only those mutants with green plant color, presumably, therefore, from R^{st} or R^{st}(light) and not from R^r, are included in Table 6. The ears from these plants were pollinated with $R^r r^g$, and r-mutant/r^g kernels were examined under 20× magnification. Occasional kernels from three mutants showed a very light spotting, and thus may be mutations to a very light stippled form. Six other mutants, represented by one ear each, gave colorless kernels only. It would not be unexpected if, on examination of larger samples of kernels of this latter group of mutants, some, or even all, showed occasional spots of aleurone color.

The colorless mutants with red plant color were presumed to be mutations from R^r. The frequency of these mutations is shown in Table 7. The ears produced by the plants grown from these mutants were pollinated with $R^r r^g$, and r-mutant/r^g kernels were examined under 20× magnification. All were found to be colorless.

The colorless and near-colorless mutants were made heterozygous with R^r, and tested for their paramutagenic action. The results of these tests are shown in Table 8. It is seen that the colorless and near-colorless mutants with green plant color are all paramutagenic, and that the colorless mutants with red plant color may be either paramutagenic or non-paramutagenic.

DISCUSSION

R^{st}(light) kernels from $R^r R^{st}$ and $R^{st} r^r$ heterozygotes were shown to result from crossing over between the R locus and a modifier about six crossover units

TABLE 7

Frequency of mutation of R^r *to* r^r *when heterozygous with* R^{st} *and* R^{st} *(light)*

Genotype	Number of kernels R^r	Number of kernels r^r	No. of r^r mutations per 10^4 gametes tested
$R^r R^{st}$	10,797	8	7.4
R^r/R^{st}(light)	4,632	2	4.3

TABLE 8

Results of the tests of the paramutagenic action of the colorless and near-colorless mutants from homozygous stippled and heterozygous R^r*/stippled ears*

Source	Number of mutants: Paramutagenic	Number of mutants: Non-paramutagenic	Total no. of mutants
Mutants with green plant color			
R^{st}(light)/R^{st}(light)	1	0	1
$R^r R^{st}$	6	0	6
R^r/R^{st}(light)	2	0	2
Mutants with red plant color			
$R^r R^{st}$	5	3	8
R^r/R^{st}(light)	0	2	2

distal to *R*. The modifier which conditions the R^{st} phenotype was designated M^{st}, and the one which conditions the R^{st}(light) phenotype was designated m^{st}. (For convenience in discussion R^{st} will be used to indicate the stippled gene at the *R* locus, and, when referring to phenotypes, to indicate the expression of an R^{st} gene accompanied by M^{st}. R^{st}(light) will be used to indicate the phenotypic expression of an R^{st} gene accompanied by m^{st}.) The expression of R^{st} is a function of the modifier that is present; R^{st} and R^{st}(light) are assumed to be identical at the *R* locus. It is possible that R^{st} and R^{st}(light) differ in the presence and absence of a single element, i.e. m^{st} may merely be the absence of M^{st}; some evidence favoring such an interpretation will be discussed later.

LAUGHNAN (1952), in a brief note, described an *r*-mutable allele (*r-m*) which produced colored spots on a colorless background. He observed that *R/r-m* plants gave rise to exceptional kernels with a much reduced frequency of colored spots. By using the proximal marker *g* and a distal translocation (T9–10a) he was able to show that the exceptional kernels were associated with crossing over between *g* and the translocation. He hypothesized that the different expressions of *r-m* were the function of a linked modifier located about four crossover units from *r-m*. These observations are similar to those reported here. It is possible that *r-m* is the same *R* allele as the one designated R^{st} in these studies. The linked modifier found in these studies to be about six crossover units distal to the *R* locus is very likely the same one reported by LAUGHNAN to be about four crossover units from *r-m*.

It is clear that an R^{st} gene modified by M^{st} gives a different phenotypic expression than one modified by m^{st}. The question might be asked whether M^{st} or m^{st} have any other effect on the R^{st} gene in addition to modification of the phenotype. A possible additional effect of M^{st} and m^{st} would be to change the mutability of the R^{st} gene. A test for a change in the mutability of R^{st} can be made by comparing the mutation rates of R^{st} and R^{st}(light). Mutations of R^{st} and R^{st}(light) to R^{sc} occurred with the same frequency in $R^{st}R^{st}$ and R^{st}(light)$/R^{st}$(light) stocks, and in $R^{st}r^{r}$ and R^{st}(light)$/r^{r}$ stocks (Table 5). This was true even though the mutation rate in the homozygous combinations was about four times greater than the rate in the heterozygous combinations. Also, the rate of mutation of R^{st} and R^{st}(light) to colorless or near-colorless was the same in $R^{r}R^{st}$ and R^{r}/R^{st}(light) heterozygotes (Table 6).

The action of the modifier is clearly not evident in terms of germinal mutations of R^{st} to R^{sc}. The action of the modifier, however, markedly effects the amount of spotting in the endosperm. If the spots in the endosperm are actually mutations of R^{st} to R^{sc} in somatic tissue, as appears likely from the fact that sectors of R^{sc} tissue have been observed to occur rarely on stippled ears, it must be assumed that the modifier acts differentially on mutation either in time or in different tissues. Possibly the modifier is "tissue limited" in its action.

The experiment designed to identify an r^{r} M^{st} chromosome also gave evidence that the modifying actions of M^{st} and m^{st} on endosperm phenotype are interchromosomal as well as intrachromosomal. The phenotypes of R^{st} m^{st}/r^{r} M^{st}/r^{r}

M^{st} and R^{st} m^{st}/r^g m^{st}/r^g m^{st} endosperms are different and easily distinguishable, even though the modifier carried on the R^{st} chromosome is the same in both compounds. The two doses of M^{st} carried on the r^r chromosomes in the first compound condition a darker expression of R^{st} than the two doses of m^{st} on the r^g chromosomes in the second compound. It was pointed out that R^{st} and R^{st}(light) show dosage effects in combinations with r (Figure 1). The above results indicate that the expression of R^{st} is a function of the dosages of M^{st} and m^{st} as well as of R^{st} and r. Changes in dosage of the factors at these two loci make 12 combinations possible, ranging from R^{st} m^{st}/r m^{st}/r m^{st}, the lightest, to R^{st} M^{st}/R^{st} M^{st}/R^{st} M^{st}, the darkest.

The data presented in Table 1 from the two heterozygous combinations (R^rR^{st} and $R^{st}r^r$) show the frequency of R^{st}(light) kernels resulting from crossing over between the R locus and a modifier of R^{st} about six crossover units distal. The five R^{st}(light) mutations from homozygous R^{st} cannot reasonably be explained as crossover products. The event necessary to produce an R^{st}(light) kernel in a homozygous R^{st} stock is a change of M^{st} to m^{st}. The change of M^{st} to m^{st} could result from mutation or crossing over. An explanation based on crossing over would require that when two M^{st} elements pair a crossover is possible that would yield m^{st} plus an unidentified complementary product. Several gene models of M^{st} could be proposed which would afford this possibility. All are basically similar, however, in that the M^{st} gene must be assumed to be bipartite, and that the two components are separable by unequal crossing over. Any hypothesis attempting to relate the change of M^{st} to m^{st} to crossing over encounters serious difficulty in explaining the two changes of M^{st} to m^{st} that occurred in somatic tissue. If crossing over is the mechanism of change, then somatic changes must be explained as instances of somatic crossing over. Somatic crossing over has been shown to occur in Drosophila (STERN 1936) and probably in certain microorganisms (PONTECORVO *et al.* 1954; JAMES and LEE-WHITING 1955; ROMAN 1956). JONES (1937, 1941) made extensive studies of somatic segregation in maize. Certain aleurone mosaics were observed which could be interpreted as resulting from somatic crossing over, but there is no genetic evidence that somatic crossing over occurs in maize (JONES 1941). Mutation as the mechanism of change of M^{st} to m^{st} does not encounter any of the difficulties associated with an explanation based on crossing over.

The data presented in Table 4 on the frequency of mutation of R^{st}(light) to R^{st}, in the light of what is now known about the genetic difference between R^{st} and R^{st}(light), are seen to be a measure of the mutation of rate of m^{st} to M^{st}, rather than a measure of the mutability of an R allele. This is true in both the homozygous and heterozygous combinations, since R^r and r^r both carry m^{st}. The total number of gametes tested was 34,214 and no mutations of m^{st} to M^{st} were found.

The R^r and r^r chromosomes in the W22 sublines both carried m^{st}. Three other inbred dent corn strains, W8, W23, and 4Co63, were tested to determine which modifier of R^{st} was present. All three were found to carry m^{st}. W8 and 4Co63

are r^r and W23 is r^g. The only proven source of M^{st} to date, therefore, is the R^{st} chromosome.

Evidence is available suggesting that M^{st} is transposable. Numerous data on transpositions of genetic material in maize have been obtained and will not be reviewed here (see McCLINTOCK 1956a, 1956b). The evidence presently available, for transposability of M^{st} is based on the ears from two plants. Numerous self-colored kernels were selected after the following cross: $R^{st}r^g \times r^gr^g$. These kernels were grown out to verify the presumed mutations of R^{st} to R^{sc}. The ears produced by the resulting plants were pollinated with r^rr^r. As observed in an earlier test, less than half of the phenotypically self-colored kernels in this experiment gave R^{sc} offspring. Fifty plants, in fact, grown from 64 self-colored kernels did not give germinally transmissible R^{sc} mutants, but segregated stippled and colorless kernels. Among these plants two were found that segregated 1/4 R^{st}, 1/4 R^{st}(light), and 1/2 r, instead of the expected 1/2 R^{st}, and 1/2 r kernels.

An explanation which satisfactorily accounts for the observed ratio would assume that M^{st} is a transposable element, and that in the two exceptional ears M^{st} has shifted from its standard position, 5.7 crossover units distal to the R locus, to a new position which assorts independently of R. m^{st} would be assumed to represent the null condition, i.e. the absence of M^{st}. Verification of this explanation of the two exceptional ears can be established by progeny tests of the three classes of kernels in question.

The information currently available concerning M^{st} and m^{st} can be logically interpreted on the assumption that M^{st} is a transposable unit, and that m^{st} is merely the absence of M^{st}. Five changes of M^{st} to m^{st} (R^{st} to R^{st}(light)) were observed in 18,586 gametes. These changes were referred to earlier in the paper as mutations of M^{st} to m^{st}. They can just as readily be explained as losses from the genome of M^{st}. The failure to observe any mutations of m^{st} to M^{st} (R^{st}(light) to R^{st}) in the 34,214 gametes tested lends additional support to a transposable unit interpretation. Had any mutants of this kind been observed a transposable unit interpretation would have required that they be explained as instances of M^{st} arising *de novo*.

Further consideration of the possible transposability of M^{st} may be deferred pending completion of a progeny test of the kernels from the two exceptional plants described above.

The data presented in Table 5 show the rate of mutation of R^{st} and R^{st}(light) to R^{sc}. The reason for the highly significant difference between the mutation rates to R^{sc} in the homozygous and heterozygous combinations is not apparent at this time. Two possibilities suggest themselves: crossing over and allelic interaction. A hypothesis based on crossing over would assume that mutations of R^{st} to R^{sc} are associated with crossing over, and that in $R^{st}r^r$ heterozygotes the frequency of such crossovers is reduced. A test was made to determine whether mutations to R^{sc} in $R^{st}r^r$ heterozygotes were associated with crossing over proximal to the R locus. Golden (g), 14 crossover units proximal to R, was used as a marker in this test. Fifteen R^{sc} mutants were obtained from an appropriate cross and the distribu-

tion of the golden marker was normal: 12 noncrossovers to 3 crossovers. Mutations of R^{st} to R^{sc} in $R^{st}r^r$ heterozygotes, therefore, are not regularly associated with crossing over proximal to the *R* locus. No test has been made of the association of R^{sc} mutations with crossing over distal to the *R* locus. One observation, however, weakens any hypothesis based on crossing over. Three $R^{st}r$ ears have been found which showed somatic sectors of $R^{sc}r$ tissue. The sectors can only be explained as the result of a somatic mutation of R^{st} to R^{sc}. A hypothesis based on the association of R^{sc} mutation with crossing over would have to assume somatic crossing over to explain the R^{sc} ear sectors. The difficulties encountered on this assumption were mentioned earlier.

A hypothesis based on allelic interaction implies that the mutability of the R^{st} gene is modified by homozygosity and heterozygosity for this allele. Similar genic interactions have been shown to occur at the reddish-*a* locus in *Drosophila virilis* (DEMEREC 1928, 1941), and at the *P* locus in maize (WOOD and BRINK 1956; BRAWN 1958). The interactions in these cases have an effect opposite from the one at the *R* locus in that mutability was greater in heterozygous than in homozygous plants.

The data presented in Table 6, showing the frequency of mutation of R^{st} and R^{st}(light) to colorless and near-colorless, call for further comment. The mutation rates reported are based on colorless and near-colorless mutants with green plant color. It was assumed that mutants with green plant color from the heterozygous combinations with R^r were mutations from R^{st}. This view is substantiated by the results of the tests of the paramutagenic action of the mutants (Table 8). All colorless and near-colorless mutants with green plant color from R^rR^{st} and R^r/R^{st}(light) heterozygotes were found to be paramutagenic, indicating R^{st} as the most likely parent allele.

No test has been made to determine whether colorless and near-colorless mutants with green plant color from R^rR^{st} and R^r/R^{st}(light) heterozygotes are associated with crossing over proximal to the *R* locus. By using the linked modifier of stippled, however, a test can be made of the association of such mutations in R^rR^{st} heterozygotes with crossing over distal to *R*. The cross from which the mutants were selected was: $R^rm^{st}/R^{st}M^{st} \times r^gm^{st}$. The modifier can be used as a distal marker for the *R* locus, and the colorless and near-colorless mutants can be tested to determine whether they carry M^{st} or m^{st} by pollinating plants carrying them with homozygous R^{st}(light). The expression of one dose of R^{st} with two doses of M^{st} carried on *r* chromosomes is darker than the expression of one dose of R^{st} with two doses of m^{st} carried on *r* chromosomes (see above). The six colorless and near-colorless mutants with green plant color from R^rR^{st} heterozygotes were tested, and all were found to carry M^{st}; these mutations, therefore, are not regularly associated with crossing over distal to the *R* locus.

Since the colorless and near-colorless mutants with green plant color from the heterozygous combinations in Table 6 are most likely mutations from R^{st}, it must be assumed that the mutability of R^{st} is greatly increased by heterozygosity with R^r. If the data in Table 6 from the two homozygous combinations ($R^{st}R^{st}$ and R^{st}(light)/R^{st}(light)) are pooled, and the data from the two heterozygous

combinations (R^rR^{st} and R^r/R^{st}(light)) are pooled also, the frequency of mutations of R^{st} to colorless and near-colorless is $0.2/10^4$ R^{st} gametes in the homozygote, and $5.1/10^4$ R^{st} gametes in the heterozygote. The 25–fold difference between the two mutation rates is the effect of heterozygosity for R^r on the mutability of R^{st} to colorless or near-colorless.

The data presented in Table 7 show the frequency of mutation of R^r to r^r. It was assumed that colorless mutants with red plant color from the heterozygous combinations would be mutations from R^r. The results of the test for paramutagenicity of the r^r mutants, however, brings into question the general validity of this assumption. Five of the ten r^r mutants exhibited a paramutagenic action on R^r in R^r/r^r-mutant heterozygotes, and the other five did not (Table 8). Evidently the r^r mutants are not a homogeneous class.

The eight r^r mutants from R^rR^{st} can be tested for their association with crossing over distal to the R locus by means of the test described above for the colorless and near-colorless mutants with green plant color. The results now available from these tests show that six of the r^r mutants carry M^{st} from the R^{st} chromosome; the other two mutants have not yet been adequately tested. Of the six r^r mutants that carried M^{st} four were paramutagenic and two were non-paramutagenic.

These facts suggest some kind of genetic recombination as the mechanism of origin of the r^r mutants in question. The r^r mutants selected from R^rR^{st} heterozygotes share some property or properties of both the R^r and R^{st} parent alleles. All have red plant color, which, presumably, can have come only from R^r; some are paramutagenic, a property that can have come only from R^{st}; six of a possible eight mutants carry M^{st} from the R^{st} chromosome. A conventional crossover mechanism that would satisfactorily explain these results must assume that a recombination is possible that will yield a chromosome carrying the plant color component of R^r, and not the seed color component of either R^r or R^{st}, but may or may not carry the paramutagenic component of R^{st}, or a marker from the R^{st} chromosome distal to the R locus. Gene models that can satisfactorily account for the observed mutants are necessarily complex, and require assumptions for which there is yet no evidence. Also, there is some question whether the paramutagenic action of R^{st} resides in a discrete unit subject to recombination in the conventional sense. This evidence comes from a study of the paramutagenic action of self-color mutants (R^{sc}) from R^{st} (Brink 1958, and unpublished). R^{sc} mutants exhibit paramutagenic actions that vary widely from the strong action characteristic of R^{st} to seemingly no action at all. Thus, paramutagenic action varies quantitatively. The transfer of paramutagenic action from R^{st} to some of the r^r mutants may possibly have occurred via some mechanism other than ordinary recombination, transposition for example. These considerations make it unprofitable at this time to set forth gene models of R^r and R^{st} seeking to explain the observed results in terms of conventional recombination only.

SUMMARY

Previous investigations have disclosed that the aleurone pigment-producing action of the R^r gene (self-colored aleurone) among the offspring of R^rR^{st}

(heterozygous stippled) plants invariably is markedly reduced. This effect has focused attention on the nature of the stippled allele and of a variant phenotype termed light stippled (R^{st}(light)).

It has been shown in the present study that R^{st}(light) differs from R^{st} only in a modifier located 5.7 crossover units distal to the R locus. The modifier affects the frequency of colored spots in the aleurone layer of the endosperm. $R^{st}r^r r^r$ endosperms averaged 20.3 spots per 5 mm^2, and R^{st}(light)$/r^r/r^r$ endosperms averaged 1.8 spots per 5 mm^2.

R^{st} and R^{st}(light) in homozygotes were observed to mutate to self-color (R^{sc}) at the respective rates of 17.0 and 19.9 per 10^4 gametes tested. When R^{st} and R^{st}(light) were heterozygous with r^r (colorless aleurone), however, they mutated to R^{sc} at the respective rates of only 4.9 and 4.3 per 10^4 gametes tested. The basis for the difference in rate of mutation of R^{st} and R^{st}(light) to R^{sc} in homozygotes and heterozygotes with r^r is not known. Several somatic mutations of R^{st} to R^{sc} were found, which indicates that mutations to R^{sc} are not regularly associated with crossing over.

R^{st}(light) in homozygotes was observed to mutate infrequently to colorless or near-colorless aleurone. No mutations of R^{st} to colorless or near-colorless aleurone were observed in R^{st} homozygotes. Mutations to colorless and near-colorless aleurorne, with either red or green plant color, were observed in both R^rR^{st} and R^r/R^{st}(light) heterozygotes. These mutants were tested for their paramutagenic action on R^r with the following result: all eight colorless and near-colorless mutants with green plant color were paramutagenic; five colorless mutants with red plant color were paramutagenic, and five were non-paramutagenic.

The colorless and near-colorless mutants with green plant color from R^rR^{st} and R^rR^{st}(light) individuals were shown to have more likely come from the stippled allele than from the R^r allele. The frequency of such mutations was found to be much higher in the R^rR^{st} and R^r/R^{st}(light) heterozygotes than in the $R^{st}R^{st}$ and R^{st}(light)$/R^{st}$(light) homozygotes. The basis for the effect of homozygosity and heterozygosity on the frequency of mutation of R^{st} and R^{st}(light) to colorless or near-colorless has not been disclosed.

The colorless mutants with red plant color were shown to have probably arisen from some type of genetic recombination between the R^r and R^{st} alleles. The mechanism of this recombination is not known; conventional crossing over or transposition, or both, may be involved.

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THE GENETIC BASIS OF NATURAL VARIATION. I. CROSSVEINS IN DROSOPHILA MELANOGASTER[1]

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AN understanding of natural variation is basic to the understanding of evolution and, more particularly, to the evolutionary potential of a group of individuals. In this regard, it is essential to understand the genetic basis of natural variation. One question on this subject stands out as important and approachable: what proportion of natural variation has a single-gene basis and what proportion has a polygenic basis?

In wild populations, it is easy to find examples of both. For example, SPENCER (1957) has isolated rare flies bearing any one of a number of genes, well known to the student of Drosophila genetics, which cause striking deviant phenotypes. On the other hand, LERNER (1954) describes many cases of what he calls "phenodeviants"—rare but widespread deviant phenotypes resulting from certain polygenic combinations. Each example leads to a different outlook as to the localization of potential variation. If one individual out of a thousand differs phenotypically because of a rare gene, then a small sample of the population would probably not have that gene, and the progeny would be bereft of the phenotype in question until a new mutation came along. On the other hand, if the one individual out of a thousand differed because of a rare combination of common genes, then even a small number of individuals, when allowed to produce a large number of progeny, would probably produce one with a deviant phenotype. The application of these alternative genetic mechanisms to human populations and all populations is clear; and since polygenic systems certainly have more than trivial importance, it is of interest to study some. Evaluation of the role of polygenic systems can come only after the thorough investigation of some examples.

Few genetic analyses indeed have ever been made on polygenic systems to the extent of localizing the component genes and characterizing them beyond their participation in the group effect. BARIGOZZI (1956) and WADDINGTON (1954) have attempted such analyses, on genes affecting tumors and crossveins respectively in *Drosophila melanogaster*. The work to be discussed here is also such an attempt (MILKMAN 1956).

[1] Most of this paper is taken from a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of Biology at Harvard University.

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This polygenic system affects the ability of *Drosophila melanogaster* to form posterior crossveins during adult development. It is similar to the crossveinless polygenes studies by WADDINGTON; in fact, use of these genes (here called *cve*) for study stems from the successful repetition of WADDINGTON's phenocopy-selection experiment. In such an experiment, one selects for reduced crossvein-making ability, as manifested by low resistance to disturbance of crossvein formation by heat shock. When one selects far enough, one begins to get a small percentage of flies that can't even make perfect crossveins in the absence of heat shock. These flies, selected further, may ultimately produce a true-breeding, polygenic, crossveinless strain. Figure 1 illustrates the character "crossvein-making ability", amount of heat shock, and resultant phenotype as a nomogram.

MATERIALS AND METHODS

In addition to the crossveinless strain isolated as described above and various marker strains, an inbred Oregon R, propagated by virgin pair matings was valuable in this work. Flies were raised under usual conditions, temperature being maintained at 25°C±1 unless otherwise noted. Special techniques will be discussed as they become relevant.

The heat shock used to induce crossvein defects was administered by immersing shell vials with pupae on their walls in a Precision constant temperature water bath at 40.5°±0.03°C. The vials were stoppered, and an air tube rose above the water surface. Moist cotton was kept in the vials to keep the pupae from desiccating during and after the treatment. Thermocouple records and comparison of twin treatments with single treatments both showed the warm up time to be exactly five minutes. Experiments on the temperature coefficient of the heat phenocopying (MILKMAN, in press) showed that time spent warming up produced no significant effects; it was therefore ignored. The animals were collected as white prepupae at the time of puparium formation. Successive collections shortened the age variation to about half an hour, generally. Treatment came 21 hours later.

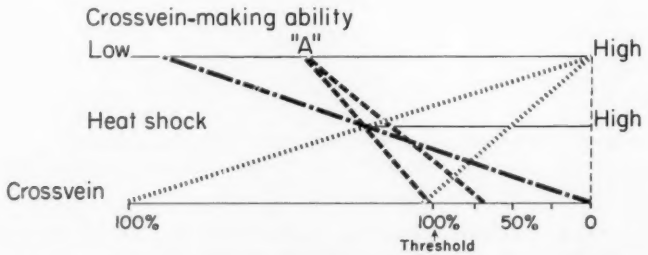

FIGURE 1.—The relationship of crossvein-making ability, heat shock during the sensitive period, and the completeness of the posterior crossveins is shown as a nomogram. For example, a fly with intermediate ability ("A") might make normal crossveins under normal conditions but defective ones in response to even slight heat shock.

EXPERIMENTAL RESULTS

The crossveinless strain: The true-breeding crossveinless strain under discussion was selected in the manner described in the introduction. It differed from wild strains in several ways. First and foremost, the posterior crossveins were, on the average, about three fourths missing in each fly. This character showed little variability from fly to fly. In addition, viability was low, judging from observations of general activity, mating behavior, life span, and sensitivity to ether and carbon monoxide. Male genitalia were often rotated, sterility was frequent among members of both sexes, and egg survival was always below 50 percent. Unless humidity was kept close to 100 percent, *cve* larvae pupated in the food instead of on the side of the vial or bottle. Finally, the body and wings were a bit stockier than those of wild type, resembling more the dachs phenotype. These other phenotypic traits may stem from the presence of the *cve* genes or other genes fixed during selection. Nothing further was done with these observations.

In order to carry out a genetic analysis of the strain, the phenotype had to be characterized in quantitative and objective terms. Accordingly, a system of rating each wing as to its posterior crossvein was developed in which the highest reliable degree of visual resolution was taken into account. It was found practical to use a scale from 0 to 6, 6 being total absence of crossvein and 0 being a perfect crossvein. The rating of the wing, then, gave the fraction (in sixths) of crossvein absent. To rate the fly, its two wing ratings were added, making possible a range from 0 (normal) to 12 (complete absence of posterior crossveins). Similarly, a population could be rated by taking an average.

The crossveinless strain averages 9. Although the location of the missing fraction was not taken into account, this strain almost without exception displayed a single small fragment of crossvein on each wing, close to or connected with the fifth longitudinal vein. The expression and penetrance of the *cve* phenotype are temperature sensitive. This can best be demonstrated in *cve*/ + flies, which exhibit more frequent and greater defects at 18°C than at 25°C. The sensitive period is coextensive with the period during which pupae are sensitive to disturbance of crossvein formation by heat shock at 40.5°. In animals raised at 25°, this period was roughly from 13 to 25 hours after puparium formation.

The genetic analysis of this strain began with two preliminary questions:

1. Is a balanced lethal system present? Crossover tests with multiple marker stocks on each of the major chromosomes showed no evidence of inversions in the *cve* stock. Salivary chromosome smears substantiated this conclusion. Therefore we can conclude that the stock was homozygous for all its *cve* genes.

2. Is there a polygenic basis for the *cve* phenotype? The rarity of crossveinless flies in the wild population might be construed as evidence of low frequency of a single gene, of a common gene sensitive to environmental effects on penetrance or viability, or of a polygenic complex.

Early attempts to obtain a true-breeding stock by pair mating the rare cross-

veinless individuals indicate that the first possibility may be discarded, since such matings produced on the average not much greater percentage of crossveinless offspring than was to be found in the wild stock itself (see Table 1).

The response to selection, on the other hand, is clear evidence for the existence of numerous genetic factors. This response manifested itself in the three ways already discussed: susceptibility to phenocopy induction rose, frequency of crossveinless flies increased, and expression also increased.

To further demonstrate the polygenic nature of this system, and to ascertain the degree of environmentally dependent variation, a series of crosses was made, beginning with reciprocal outcrosses to inbred "isogenic" Oregon R flies. The use of Oregon R was determined by the desire to introduce as little variation into the system as possible. Although the Oregon R stock undoubtedly differs more from the *cve* stock than does the ancestral wild stock, the obvious genetic variability of the latter seemed a difficult thing to cope with.

The results of these and subsequent crosses are given in Table 2 and are shown in graphic form in Figure 2. By the use of the rating system, it was seen that the

TABLE 1

Incidence of untreated crossveinless flies in a wild Massachusetts strain from which the cve *strain was ultimately derived*

Generations	+ Female	+ Male	*cve* Female	*cve* Male	Percent *cve* Female	Percent *cve* Male	Combined
			Cage sample				
	544	561	1	0	0.2	0.0	0.1
			Inbreeding				
1	...	..	.	.	.		..
2	277	284	0	0	0	0	0
3	742	769	5	1	0.7	0.1	0.4
			Pair matings of untreated crossveinless flies				
F_1	378	329	2	1	0.7	0.3	0.5
F_2	1010	1025	2	6	0.2	0.6	0.4

TABLE 2

Results of crosses involving cve *and Ore R.*

Parent Female	Parent Male	Designation of progeny class	Females N	Females *cve*	Females Percent *cve*	Females r	Females r′	Males N	Males *cve*	Males Percent *cve*	Males r	Males r′
Ore R	× *cve*	F_1a	816	150	18.4	0.355	1.93	769	5	6.4	0.013	2.00
cve	× Ore R	F_1b	219	27	12.3	0.228	1.85	162	18	11.1	0.172	1.55
F_1a	× *cve*	B_1a	160	110	68.7	3.44	5.01	205	75	36.8	2.15	5.88
cve	× F_1a	B_1b	200	126	63.0	3.25	5.16	231	134	58.0	3.87	4.57
cve	× B_1a or B_1b	B_2	361	306	84.8	6.03	7.12	301	232	77.1	5.76	7.46
F_1a	× F_1a	F_2	293	66	22.5	0.88	3.90	309	36	11.6	.55	4.75
		(*cve*	89	89	100	9.11	9.11	95	95	100	9.17	9.17)

r = Average rating, all flies.
r′ = Average rating, crossveinless flies.

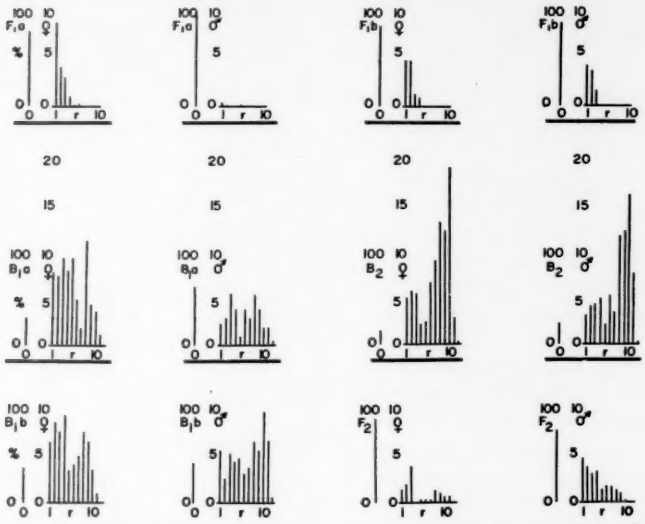

FIGURE 2.—Results of the crosses referred to in Table 2 are plotted as frequency distributions of crossvein defect ratings. Since the normal ("0") flies are usually by far the most numerous, they are plotted on a scale running from 0 to 100 percent. The other classes, representing various degrees of crossvein defects, are on a scale from 0 to 10 percent or 0 to 20 percent. Further explanation in text.

results were characteristic of a polygenic complex, in that repeated backcrosses brought the average crossvein defect up toward the level of the *cve* parents. The distinction between accumulation of more *cve* genes per fly and higher incidence of a single gene in the class is clearly made by the increased average expression of the crossveinless individuals as the backcrosses are made.

It will be seen in Table 2 that the more *cve* genes there are, the higher the expression and penetrance of the *cve* phenotype. Furthermore, within the scope of the phenotype, the genes seem to be acting superadditively. For example, the F_1a and F_1b females should have half as many *cve* genes as the *cve* females, yet their average ratings are well below one tenth that of the *cve* females.

On the other hand, a glance at some of the distributions in Figure 2, notably those of the F_1's, gives the impression that they are truncated—artificially cut off by the limits of the rating scale. Moreover, there is a basis for believing that many of the "0" flies should be rated on the minus side of the scale; this would be rating them on the basis of their crossvein-making ability, whose units would be taken from the scale already set up for the range of visible variation, the crossvein defects (see Figure 3).

It would be well to pause long enough to set forth in some detail the implications both of the term "crossvein-making ability", and of its relation to the rating system. It is here postulated, and supporting evidence will soon be given, that

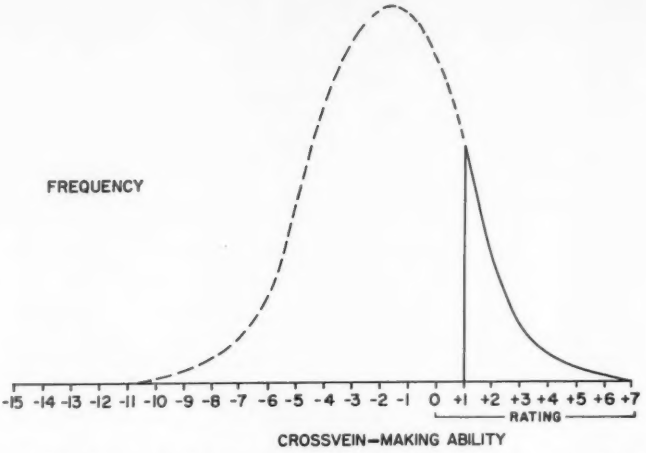

FIGURE 3.—The frequency distributions of ratings of females from *cve* × Ore R and from Ore R × *cve* crosses look like truncated portions of a normal distribution. This fact, suggests that these flies may be normally distributed with respect to a broader variable, crossvein-making ability. Conformity to such a truncated portion is demonstrated by the use of probits, as shown in Table 3 and Figure 4.

all the *cve* genes act on the same prerequisite of posterior crossvein formation. It may be the synthesis of a substance, the mobilization or alteration of cells, or any one of a number of processes; what is important to us is that the degree to which it is carried out has relevance to the ultimate form of the posterior crossvein. It is this process which is the physiological counterpart of the term "crossvein-making ability" used here.

As STERN (1943) and HOUSE (1953) have shown so clearly in their respective studies on gene interaction relating to wing venation, the structural reflection of developmental change may be a threshold phenomenon. Thus, no visible manifestation of various levels of a hypothetical substance "P" was seen until a threshold was crossed. Then vein defects appeared. The three genes, recessive and superadditive with respect to the vein defects, were shown to be semidominant and additive with respect to the underlying process which HOUSE chose to call the synthesis of "P".

A similar problem is faced with the *cve* genes. Here an attempt will first be made to delineate the norm of reaction mathematically, in order to have a way of better understanding the *cve* genes. Later, an attempt will be made to corroborate the physiological reality of this norm of reaction by an attack from a different direction.

Now, finally, one more thing must be said about the rating system before the data are presented. The rating units are set up in the simplest empirical way; they correspond to fractions of missing crossvein. But there is no reason to assume

that the amount of crossvein made is linearly proportional—threshold or no—to any underlying developmental process. That remains to be demonstrated. What is to be hoped is that in the quantitative terms of the underlying process the difference between a "1" fly and a "2" fly is the same as between a "6" and a "7".

One way of demonstrating that this is the case is to show that the variation in rating is normally distributed. This normal distribution would best be sought in the absence of genetic variation, since, if the number of genes involved were small, genetic variation would introduce several modes into the distribution.

If the rating system is internally consistent, it may be extrapolated beyond the "0" threshold. If the genes can now be shown to behave additively (with respect both to other genes and to their homologs, i.e. without dominance), then one can infer that these *cve* genes determine the norm of reaction relating to one aspect of crossvein formation.

Since the evidence is strong that the *cve* genes are homozygous, and since the Oregon R stock is highly inbred, the F_1a should have no relevant genetic variation. The normality of its rating distribution may be tested by converting frequency into probits, as is done in Table 3. These transformed data should fall on a straight line if the distribution is normal, and they do, as is seen in Figure 4. The line is extrapolated to the probit value of five, where the mean may now be measured. It is −1.75, a value for the norm of reaction of this genotype in rating units. Standard deviation is graphically estimated at 2.75 units.

If the heterozygous *cve* genes of the F_1 now segregate in a backcross or F_2, a multimodal distribution should be seen. That this happens is shown in Figure 2. For the F_1's, however, even where the data show very few crossveinless individuals, it seems reasonable to assume that the hypothetical ratings are distributed normally on the other side of the threshold, so that the norm of reaction can be calculated from the few positive ratings available.

Since the backcross data were unsuitable for probit transformation, visual extrapolations were made instead, to compensate for the truncation of the distribution. The average of the norms of reaction corresponding to the various genotypes in the backcross progeny could then be roughly estimated.

TABLE 3

Conversion of F_1a female rating frequencies into probits

Rating class	Frequency	Cumulative frequency	Percent	100—%*	Probits
7	1	1	0.12	99.88	8.04
6	2	3	0.37	99.63	7.68
5	1	4	0.49	99.51	7.58
4	9	13	1.59	98.41	7.15
3	30	43	5.26	94.74	6.62
2	34	77	9.45	90.55	6.32
1	74	151	18.50	81.50	5.90
0	665	816	100.	0	...

* This step is unnecessary but was done because the probit table used has an extra decimal place above 98 percent.

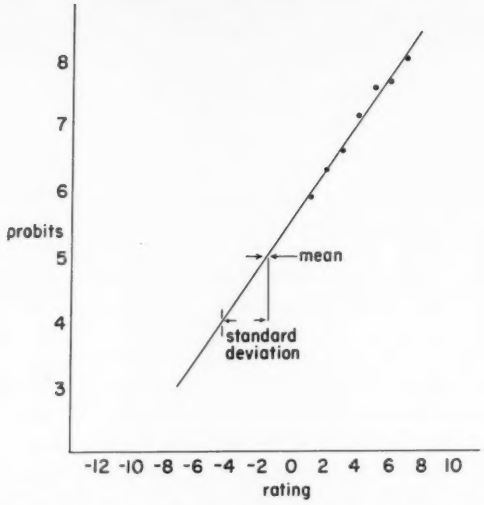

FIGURE 4.—Transformation of a normal curve into a straight line is done by converting variate units into units of standard deviation plus 5. Points obtained from similar treatment of data from F_1a females are fairly close to a straight line. The mean is where the line intersects the probit value of 5, and the standard deviation in rating units is graphically demonstrated as the horizontal deflection of the line as it moves one probit unit.

The rating of the parental *cve* stock could be computed merely by averaging individual ratings, since none of these flies were wild type. The assumption is made in doing this that here, too, the rating units are equivalent to the units lower down on the scale.

Additive action of the cve *genes:* As may be seen from Table 2, differences in the average ratings of F_1a and F_1b males suggest that a sex-linked *cve* factor is present. This fact has a bearing on the choice of data to examine in order to find out whether or not the *cve* genes are acting additively on a process underlying crossvein development. If the genes are acting additively, there should be a quantitative correlation between corrected ratings and number of *cve* genes.

From the F_1 and backcrosses, progeny classes can be chosen for comparison so that one is genetically intermediate between the other and the *cve* parents of one sex. If the corrected ratings follow the same pattern, the *cve* genes may be assumed to act additively. If this is the case, and since the F_1 females are genetically intermediate between the Oregon R females and the *cve* females, the norm of reaction for Oregon R with respect to this aspect of crossvein formation can be calculated.

Table 4 shows two series of three progeny classes each and their ratings. The middle member of each series is exactly intermediate genetically between the other two. It will be seen that the ratings follow this quantitative relationship

TABLE 4

Relation of cve *chromosomes to corrected rating*

Sex	Sets of *cve* autosomes	Sets of *cve* X chromosomes	Class	Corrected rating
f	2	2	*cve*	+ 9.11
	1½ (avg)	1½ (avg)	B_1a	+ 3.25
	1	1	F_1a, F_1b	− 2.
m	2	1	*cve*	+ 9.17
	1½ (avg)	½ (avg)	B_1a	− 1.
	1	0	F_1a	− 8.

Calculation of rating of parental Ore R flies

Sex	Sets of *cve* autosomes	Sets of *cve* X chromosomes	Class	Corrected rating
f	2	2	*cve*	+ 9.11
	1	1	F_1a, F_1b	− 2.
	0	0	Ore R	−13. (calc)
m	2	2	*cve*	+ 9.17
	1	½ (avg)	½(F_1a+b)	− 5.
	0	0	Ore R	−19. (calc)

fairly well also. We may say, then, that the *cve* genes do act additively, and the crossvein-making ability of the Oregon R flies may now be calculated. Table 4 shows the series that are used. The F_1 females, −2, are intermediate between the *cve* females, +9, and the Oregon R females. Since $+9 - (-2) = 11$, then $-2 - 11 = -13$, the value for Oregon R females. The Oregon R females, then, have 13 units of crossvein-making ability (c.m.a.) in excess of their needs.

For the males, the values of the F_1 males are average at −5. The *cve* males are +9. Since $+9 - (-5) = 14$, then $-5 - 14 = -19$, the value for the Oregon R males. The farther a c.m.a. value is from 0, the greater the reserve ability to carry on this aspect of crossvein formation. This rating difference between Oregon R males and females reflects a fact we already know, and indeed the calculated value results from it: that many fewer F_1 males than females are crossveinless.

Corroboration of the values calculated for the norms of reaction of Oregon R males and females: In order to give substance to these numbers, −13 and −19, brief mention may be made of an experiment which corroborates these estimates from an entirely different angle. Fuller discussion will come in a later paper (MILKMAN, in press).

The norms of reaction of the Oregon R flies given above were measured with respect to a certain process underlying crossvein development. If this same process could be altered by nongenetic means, a study of the relationship of treatment dose to response should enable us to calculate the same norm of reaction.

When 23-hour pupae are treated at 40.5°C, no change in adult morphology is

noted unless the treatment lasts longer than about 25 minutes. Above this threshold, response (crossvein defects) increases with duration, apparently linearly. Each sex has its own rate. Extrapolation of these curves back to zero time gives the following values for the norm of reaction. Oregon R males, −22; females, −12½. These values compare well with −19 and −13, respectively.

Roughly, then, we can quantify a physiological state, whose nature we know nothing of, except that it is one measure of a fly's ability to make posterior crossveins. The further below zero it is, the more reserves the fly had in the face of outside interference. This state is quantitatively controlled by the genes which make up the *cve* complex, and a diagram of the relationships involved is given in Figure 5.

Evidence for the multichromosomal nature of the cve *complex:* The F_1 data have already shown that *cve* factors are present both on the X chromosome and on one or more autosomes. A simple demonstration of the involvement of all three major chromosomes comes from the following experiment: *cve* females were mated to *Cy/Pm D/Sb* males. *Cy/Pm D/Sb* is a multiple inversion stock containing Curly and Plum in repulsion on the second chromosomes and Dichaete and Stubble in repulsion on the third chromosomes. These dominant markers affect wing shape, eye color, wing position, and bristles, respectively. They are all lethal when homozygous. From the above cross, as is illustrated in Table 5, several crossveinless males were taken and mated back to *Cy/Pm D/Sb* females. The crosses gave rise to progeny whose unmarked second and third chromosomes were uniform and contained whatever *cve* factors were present in the

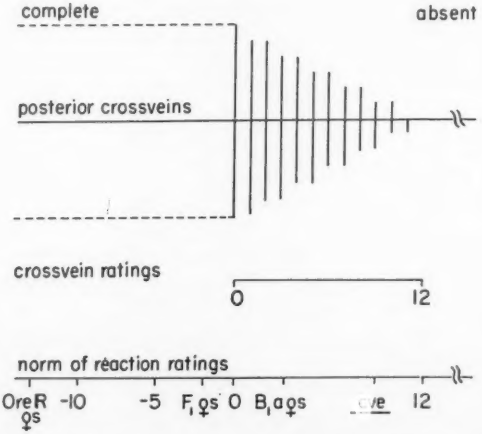

FIGURE 5.—Under normal conditions, crossvein-making ability is directly translated into crossveins. One may choose a degree of c.m.a., which is a norm of reaction, see the crossvein rating the fly would have, and see this expressed visually as the fraction of crossvein absent.

TABLE 5

The BL crosses

Original cross:	+/+	*Cy/Pm*	*D/Sb*	× *cve/*	*cve/cve*	*cve/cve*
Backcross:	+/*cve*	M/*cve*	M/*cve*	× +/	*Cy/Pm*	*D/Sb*
Final cross:	Back cross progeny			× +/	+/+	*cv-c/cv-c*

Possible genotypes

Backcross progeny			BL progeny		
Chromosome X	II	III	X	II	III
+/	M+/*cve*	M+/*cve*	+/	M/+	M/*cv-c*
+/+	M+/+M	M+/+M	+/+	*cve*/+	*cve/cv-c*
cve/			*cve*/		
cve/+			*cve*/+		

M stands for *Cy*, *Pm*, *D*, or *Sb*.

parental second and third chromosomes. Also, the females had an X chromosome from the *cve* grandmother.

It was desired to test the contribution of each of the major chromosomes by mating these flies in such a way as to permit measurement of the *cve* effect of each unmarked chromosome. The partial dominance of the *cve* genes made crossing back to the *cve* flies undesirable.

At this point, advantage was taken of the interaction of the *cve* complex with *crossveinless-c*, a completely recessive third chromosome mutant which produces a crossveinless phenotype. Evidence presented in the second paper in this series shows that the *cv-c* locus is probably not involved in the *cve* complex. The *cv-c* allele used here is certainly not. This interaction proved valuable in providing a uniform booster which enabled small numbers of *cve* genes to have a morphological effect. An illustration is given by the crosses under discussion.

When a group of Marker/ *cve* sisters or a group of brothers was mated to *cv-c* flies, the markers and *cve* genes segregated in such a way as to reveal the contribution of each chromosome to the *cve* complex. These crosses were designated BL. Table 6 lists the average ratings of the progeny of these crosses, which are not compensated for sub-zero values. It may be seen from Table 6 that each *cve* chromosome has an effect both individually and in combination with others. The contribution of the X chromosome may be seen by comparing the data for males of crosses 1, 2, and 3, whose mothers were heterozygous for the *cve* X chromosome, with the data for males of cross 4, whose mothers did not supply their *cve* factors.

The frequency distributions were not all normal, so that the probit method of estimating the true means could not be used, but the averages give some idea of the relative contributions of each chromosome. The second and X chromosomes seem equally important, the third about twice as important as either.

Surely two of the outstanding properties of the crossveinless complex are the interaction of the components with one another and the similarity of their inter-

TABLE 6

Results of BL crosses

Crosses 1, 2, 3

$$\frac{M\ +}{+\ cve}\ \frac{+M\ +}{+\ +\ cve}\ \frac{+\ +}{+\ cve} \times \frac{+\ +}{+\ +}\ \frac{cve\ +\ +}{cv\text{-}c\ +\ +}\ \frac{+}{\ }$$

50% of males get *cve* X

50% of females get *cve* X

Cross 4

$$\frac{+\ +}{+\ +}\ \frac{cv\text{-}c\ +\ +}{cv\text{-}c\ +\ +}\ \frac{+}{+} \times \frac{M\ +}{+\ cve}\ \frac{+M\ +}{+\ +\ cve}\ \frac{cv\text{-}c\ +}{\ }$$

no males get *cve* X

50% of females get *cve* X

Average ratings of BL progeny

Marker Class	Cross	50 percent with *cve* X chromosomes Female	50 percent with *cve* X chromosomes Male	None with *cve* X chromosomes Male	*cve* Autosomes	Pooled average ratings Female 1,2,3	Pooled average ratings Female 4	Pooled average ratings Male 1,2,3	Pooled average ratings Male 4*
++	1	8.36	9.00						
	2	8.19	8.57		II, III	8.19	8.17	8.62	5.50
	3	7.88	8.21						
	4	8.17		5.50					
Cy +	1	5.17	6.53						
	2	6.65	7.65		III	5.84	5.60	7.00	1.33
	3	4.00	5.60						
	4	5.60		1.33					
Pm +	1	5.61	6.72						
	2	6.28	6.54		III	5.84	5.61	6.72	1.73
	3	5.64	7.05						
	4	5.61		1.73					
+ *D*	1	...	...						
	2	0.11	0.89		II	0.42	1.00	1.00	0.40
	3	1.50	3.00						
	4	1.00		0.40					
+ *Sb*	1	2.36	6.15						
	2	0.94	2.40		II	2.06	...	4.32	...
	3	3.32	7.23						
	4	...	...	...					
Cy D	1	0	0.65						
	2	0.08	0.48		..	0.03	0.57	0.58	0
	3	0	0.67						
	4	0.57	...	0					
Pm D	1	0.04	0.39						
	2	0.24	0.24		..	0.10	0.13	0.39	0
	3	0.00	0.75						
	4	0.13	...	0					
Cy Sb	1	0.13	0.52						
	2	0.42	1.81		..	0.25	...	1.16	...
	3	0.13	1.36						
	4	...	...	...					
Pm Sb	1	0.26	0.94						
	2	0.20	1.14		..	0.22	0.50	1.18	0
	3	0.19	1.66						
	4	0.50		0					

* The cross 4 males carry no *cve* X chromosomes.

The average crossvein defect rating of a class of flies shows the influence of each of the major chromosomes

actions with *cv-c*. For, in examining the results of the BL crosses, Table 6, one sees the two lines of evidence that there are *cve* genes in each of the major chromosomes. One is that in classes with any one of these three chromosomes unmarked, one finds some crossveinless flies. And the other is that if one compares any two classes differing only by one marked chromosome, no matter which, the class with the extra marked chromosome has the lower average rating. This may also be seen in crosses not involving *cv-c*. Table 7 lists the percentages of crossveinless flies in the results of an early experiment done before ratings were used.

The above approaches to the *cve* complex have not been of the type likely to result in information about the individual genes. Having found some of the general facts—that the genes are on all three major chromosomes, that they are semidominant and act additively in the determination of a state underlying crossvein formation—it would now be well to consider two specific approaches to the more precise characterization of the genes involved. These are (1) interaction with genes known to cause crossvein defects, and (2) crossover tests. Results of these approaches will be discussed in the next paper in this series (MILKMAN, in press).

Discussion of the results presented here will be deferred to the next paper of this series.

TABLE 7

*Results of inbreeding Marker/*cve *heterozygotes*

(*cve*) *Pm/+* *Sb/+* × (*cve*) *Cy/+* *D/+*

Grouped by second chromosome marker classes

Second chromosome	1. + F	1. + M	1. *cve* F	1. *cve* M	2. + F	2. + M	2. *cve* F	2. *cve* M
Cy/Pm	9	8	17	13	6	4	0	1
Cy/+	7	10	21	18	4	1	6	7
+/Pm	8	7	22	17	5	4	3	2
+/+	0	0	0	0	0	1	2	1

Grouped by third chromosome marker classes

Third chromosome	1. + F	1. + M	1. *cve* F	1. *cve* M	2. + F	2. + M	2. *cve* F	2. *cve* M
D/Sb	17	17	3	3	10	4	0	0
D/+	2	5	20	17	1	0	3	1
+/Sb	2	3	21	18	4	6	2	7
+/+	0	0	16	10	0	0	6	3

SUMMARY

1. A true-breeding stock with polygenically caused posterior crossvein defects has been selected from a wild strain of *Drosophila melanogaster*.

2. The genes which cause crossvein defects also increase susceptibility to crossveinless phenocopying by heat.

3. The polygenes are on all three major chromosomes.

4. A character underlying posterior crossvein formation and affected by the crossveinless polygenes is defined as crossvein making ability. In terms of it, the polygenes can be seen to be acting additively. The reserve crossvein making ability (in excess of the normal need) can be measured both by analysis of crosses and by analysis of phenocopy dosage-response data.

5. The use of "booster" genes to render detectible genes with quite small effects is discussed.

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STREPTOMYCIN RESISTANCE IN ESCHERICHIA COLI ANALYZED BY TRANSDUCTION[1]

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TWO main categories of mutation affecting bacterial susceptibility to the action of streptomycin have been described and studied, streptomycin resistance and streptomycin dependence (MILLER and BOHNHOFF 1947; DEMEREC 1948, 1950, 1951; DEMEREC *et al.* 1949; NEWCOMBE and HAWIRKO 1949; NEWCOMBE 1952; USHIBA and WATANABE 1954; WATANABE 1954, 1955). Resistance to a high concentration of streptomycin develops either in a single mutational step or by multiple steps. Genetic differences between mutants of the single-step type and the multistep type have been demonstrated by means of transformation experiments with *Diplococcus pneumoniae* (HOTCHKISS 1952; HASHIMOTO 1955a, 1957), recombination experiments with *Escherichia coli*, strain K-12 (USHIBA *et al.* 1957), and transduction experiments with *Salmonella typhimurium* (WATANABE and WATANABE, in press, a,b). Streptomycin-dependent mutants can be obtained in a single mutational step. They may differ from one another with regard to concentration of the drug required, ability to satisfy the streptomycin requirement with certain related chemicals, and some other characteristics (DEMEREC *et al.* 1949; SZYBALSKI and COCITO-VANDERMEULEN 1958). Genetic analysis by recombination experiments with *E. coli* K-12 has shown that independently originating dependent mutants are the result of mutations within a single gene locus or in very closely linked loci (DEMEREC *et al.* 1949; NEWCOMBE and NYHOLM 1950; SZYBALSKI and COCITO-VANDERMEULEN 1958), and that single-step high resistance is also inherited as if controlled by alleles of the same gene locus (NEWCOMBE and NYHOLM 1950). In transduction experiments with *E. coli* a similar observation was made (LENNOX 1955). These results indicate the presence of a particular gene locus concerned with single-step streptomycin resistance and dependence, although a number of other loci may be responsible for multistep resistance.

The study described here attempted to answer two questions: (1) whether or not streptomycin-dependent and single-step highly resistant mutants of independent origin do result from mutations at the same gene locus; and (2) if so, whether the mutations occur at different sites of the locus—that is, whether nonidentical allelism can be demonstrated in this case as it has been by DEMEREC

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and his collaborators in the case of auxotrophic mutants of *S. typhimurium* (DEMEREC *et al.* 1955; DEMEREC and Z. DEMEREC 1956; DEMEREC and Z. HARTMAN 1956; DEMEREC 1956a,b; HARTMAN 1956).

It proved unexpectedly difficult to obtain recombination between streptomycin-dependent mutants, which suggested that they might be the result of multisite changes. As such, they would not be expected to revert to wild type. Mutants having the wild type phenotype were in fact easily obtained, however, and so the next step in the study was analysis of their genotypes to ascertain whether they were true revertants, due to back mutation at the same locus, or the result of change in some other part of the genome. Reports of genetical analyses of such "revertants" by transformation experiments with *D. pneumoniae* (HASHIMOTO 1955b and unpublished) and by recombination experiments with *E. coli* (NEWCOMBE and NYHOLM 1950) indicated that at least some of them still carried in their genomes the original streptomycin-dependent mutation.

MATERIALS AND METHODS

Bacterial strains: All strains used were *E. coli* strain B/r and its mutants. The original strain B/r will be referred to simply as *str-s* because of its sensitivity to streptomycin. All streptomycin-dependent and -resistant strains were obtained by single-step selection of spontaneous mutants from *str-s*, and are designated by the abbreviations *str-d* and *str-r*, respectively, followed by numbers indicating their separate mutational origins (e.g., *str-d-1*, *str-d-2*). Streptomycin-dependent strains came from DEMEREC's stocks (obtained by selection with 50 μg/ml of streptomycin), and *str-r* strains were isolated during the experiment by selection with 500 μg of streptomycin per ml. Responses to streptomycin of the strains mainly used are shown in Table 1.

TABLE 1

Growth of str-d *and* str-r *strains on nutrient agar containing various concentrations of streptomycin. After 24 hours of incubation, growth was scored from — (none) to +++ (very heavy)*

Strain	Concentration of streptomycin (μg/ml)							
	0	1	5	20	100	500	1000	5000
str-s	+++	+	—	—	—	—	—	—
str-d-1	—	+	++	+++	+++	++	++	+
-4	—	++	++	+++	+++	—	—	—
-5	—	++	+++	+++	+++	±	—	—
-62	—	+	++	+++	+++	++	+	—
-67	—	++	+++	+++	+++	—	—	—
-74	—	++	+++	+++	+++	—	—	—
-77	—	++	+++	+++	+++	±	—	—
-78	—	±	++	+++	+++	++	±	—
str-r-2	+++	+++	+++	+++	+++	+++	++	++
-3	+++	+++	+++	+++	+++	+++	++	++

Nondependent mutants were isolated from dependent strains by selection on medium lacking streptomycin.

Phage: The strain used chiefly in these experiments was P1bt, a turbid-plaque isolate from phage P1b derived from LENNOX's strain P1kc. The original P1 strain was isolated by BERTANI (1951a).

Preparation of phage lysates: Lysates were prepared on confluent lysis plates (see SWANSTROM and ADAMS 1951) with modified HERSHEY tris glucose medium. Prewarmed, undried tris agar (one percent agar) plates containing about 40 ml per plate were overlaid with 2.5 ml soft tris agar (0.65% agar) containing about 2×10^8 log phase bacteria from an aerated liquid tris glucose culture and about 5×10^5 phage particles. As soon as possible after hardening, the plates were incubated for about 6–7 hours. After confluent lysis, the bacteria remaining on the plates were killed with chloroform vapor; the soft agar layer was scraped and washed off with 2.5–5 ml of prewarmed eluting fluid and centrifuged at low speed. The supernatant was shaken with 1/10 volume of chloroform and bubbled with air to remove the chloroform. After storage in a cold room overnight, the lysate was centrifuged again to remove the remaining agar, and treated once more with chloroform.

The preparations were titrated, using tris agar and a phage-sensitive strain as indicator. Their sterility was verified by plating a sample on nutrient agar. Titers were usually between 5×10^9 and 3×10^{10} per ml. The lysates were stored in a cold room, usually with a small amount of chloroform, and were fairly stable for at least several months. A sample tested after six months of storage showed a decrease in titer to one fifth the original.

When lysates were made from *str-d* strains, 100 μg/ml of streptomycin was added to the medium, since it was observed that *str-d* strains require 5 to 50 times more streptomycin in the tris medium than in nutrient broth medium. Sometimes it was difficult to get high titers in phage lysates because of variations in growth among different *str-d* strains, and in the case of *str-d-4* it was necessary to add more $CaCl_2$ (about 10^{-2} M).

Transduction technique: An aerated culture of the recipient bacteria in L broth containing 2.5×10^{-3} M $CaCl_2$ was employed in transduction experiments just before it reached full growth ($1–2 \times 10^9$ bacteria per ml). In the case of *str-d* strains, streptomycin was added (10 μg/ml) and after suitable growth the culture was centrifuged and the bacteria were resuspended in L broth or tris medium without glucose.

The technique was as follows. In some experiments, equal volumes of $1–2 \times 10^9$/ml recipient bacterial culture and 1×10^{10}/ml donor phage lysate were mixed; in other experiments the proportion was 1/10 volume of $0.5–2 \times 10^{10}$/ml bacterial culture and 5×10^9/ml phage lysate. The mixture was kept at 37°C for 30 minutes to allow adsorption of the phage. Under these conditions (phage multiplicities between one and ten), it was found that more than 80 percent of the phage particles were adsorbed to bacteria, and that bacteria survival was from 35 to 55 percent. In trials made with *leu* (leucine requirement)

as a marker, the number of transductants was 50 to 80 percent of the expected number calculated on the basis of results obtained in an experiment using lower multiplicity and assuming that frequency of transduction increases lineally with number of phage particles employed. With still higher multiplicity (about 70), bacterial survival was ten percent and the number of transductants was 3.5 percent the expected number.

In a formula describing a transduction experiment, the genotype of the recipient bacteria appears first, followed by the sign (x) and the genotype of the donor bacteria. Thus: *str-d-77* (x) *str-s*.

Methods of selecting transductants: After adsorption, 0.1 ml samples of the mixture were plated. When selection was to be made without streptomycin, the samples were spread on the surface of nutrient agar. When selection was to be made with streptomycin, on the other hand, each sample was mixed with 20 ml of melted nutrient agar in a Petri dish, and incubated at 37°C as soon as possible after hardening. Usually after three hours incubation, to allow the bacteria to undergo several divisions, two ml of nutrient soft agar containing 550 μg/ml of streptomycin was added, and the plate was reincubated. The final concentration after complete diffusion of the streptomycin was 50 μg/ml. Transductants were scored after three or four days of incubation.

Media: Nutrient agar: NaCl, 1 gm; dehydrated nutrient agar, 23 gm; H_2O, 1 liter.

Soft nutrient agar: NaCl, 5 gm; dehydrated nutrient agar, 11 gm; H_2O, 1 liter.

Enriched minimal agar: K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; sodium citrate $5H_2O$, 0.47 gm; $MgSO_4$ anhydrous, 0.05 gm; $(NH_4)_2SO_4$, 1.0 gm; dehydrated nutrient broth, 0.01%; glucose, 0.2%; agar, 1.5%; H_2O, 1 liter.

L broth: tryptone, 1%; yeast extract, 0.5%; NaCl, 0.5%; glucose, 0.1%; pH, 7.0. (LENNOX 1955.)

Modified tris glucose medium: HERSHEY's tris glucose medium (1955) modified to contain 2.5×10^{-3} M $CaCl_2$.

Eluting fluid: NaCl, 0.3%; peptone, 0.1%.

Streptomycin: streptomycin sulfate.

EXPERIMENTAL RESULTS

Transduction of str-d, str-r, *and* str-s: It was noticed early in these studies that bacteria which acquire either *str-r* or *str-d* by transduction have to undergo several divisions before becoming resistant to streptomycin; that is, several divisions are required for phenotypic expression. Table 2 gives the results of one experiment designed to discover whether streptomycin dependence could be transferred by transduction, and to determine the most suitable conditions for detecting *str-d* transductants. The recipient bacteria were sensitive to streptomycin, and the donor bacteria carried the *str-d-77* marker. Plates containing the infected recipient cells were incubated for 0, 1, 3, 5, or 7 hours before streptomycin was added. The results show that *str-s* was transduced to *str-d-77*, and

TABLE 2

Delayed appearance of str-d *transductants. Number of* str-d *transductants obtained when streptomycin was added at different times after incubation of the bacteria*

	Streptomycin added after (hours)				
	0	1	3	5	7
str-s (x) *str-d-77*	0	3	53	29	0
Control	0	0	1	1.5	0

TABLE 3

Transduction experiments with str-d, str-r, *and* str-s *markers*

Recipient	Donor	Colonies per plate*	
		Treated	Control
str-s	*str-d-4*	58	1
"	*-5*	142.3	1
"	*-62*	18	1
"	*-67*	29	1
"	*-74*	380	1
"	*-77*	223.1	0.9
"	*str-r-2*	50.5	0.5
"	*-3*	116	0.5
str-d-1	*str-s*	12	3
-4	"	36	9
-5	"	241	26
-67	"	264	22
-77	"	307	35
-78	"	266	141

* Average from two to ten plates.

that the largest number of transductants was recovered after a 3-hour incubation. Presumably the shorter incubation period was not sufficient for phenotypic expression of the dependence, and longer periods allowed the background growth of sensitive bacteria to become so heavy that a considerable proportion of transductant cells were not able to form colonies. A similar method was found effective for the transfer of resistance from *str-r* donors to sensitive recipients (see Table 3).

It is possible to detect transduction to *str-s* in *str-d* recipients. In this case the phage infected bacteria are plated on medium lacking streptomycin, where non-transductant (*str-d*) cells can undergo only a few divisions but transductants, as well as "revertants," are able to form colonies. The number of divisions undergone by *str-d* bacteria in the absence of streptomycin (residual growth; BERTANI 1951b), is characteristic for each independently originating *str-d* mutant. The data of Table 3 show that *str-s* transductants were detected when any one of six *str-d* strains was the recipient. Similar experiments with *str-d-3*, *-62*, *-66*, *-69*, *-70*, *-71*, *-72*, *-73*, and *-74* as recipients, however, gave negative results. Ap-

parently the residual growth of these strains is not sufficient to permit the expression of *str-s*. Even in some of the positive experiments the number of *str-s* transductants was small, presumably because of inadequate residual growth of the recipient bacteria. Best results were obtained with *str-d-5* and *str-d-77*, and these two strains were the ones mainly used in subsequent experiments.

Transduction between str-d *mutants:* If *str-d* mutants of independent origin were the result of mutations at different, unlinked loci that could not be carried in the same transducing fragment, then transduction experiments between two such *str-d* mutants would be expected to produce as many *str-s* recombinants as experiments between the same *str-d* recipient and an *str-s* donor. On the other hand, if two *str-d* mutants were identical alleles, no recombinants would appear; and if they were either nonidentical alleles or the result of mutations at two closely linked loci, a few recombinants would be expected, the number depending on the chromosomal distance between the two markers.

Extensive experiments with *str-d-4*, *-5*, *-62*, *-67*, *-74*, and *-77* produced no detectable recombinants, thus eliminating the possibility that these mutants represent loci carried in different transducing fragments. The available method, however, was not sensitive enough to detect recombinants occurring in small numbers, as might be expected in experiments involving nonidentical alleles. Thus the results of these tests are not definitive but make it seem probable that one locus determines streptomycin dependence.

Transduction between str-d *and* str-r *mutants:* Results to be expected in experiments with *str-d* as recipient and *str-r* as donor would be quite different, depending on whether these markers are closely linked (carried in one transducing fragment) or are not closely linked. A donor marker can be recovered only in the case of close linkage, so that if *str-d* and *str-r* were not linked the results of such experiments would be similar to those obtained with the wild type (*str-s*) instead of *str-r* as donor. Table 4 shows the results of experiments in which *str-d-77* was the recipient and either *str-r-3*, *str-r-2*, or wild type the donor. Of 1826 tested transductants from an experiment in which the wild type was donor, 1825 were

TABLE 4

Results of transduction experiments with str-d-77 *as recipient and* str-r *or* str-s *mutant as donor*

	Donor			Control
	str-r-3	*str-r-2*	Wild type	
Number of experiments	3	2	1	2
Number of plates	53	40	10	40
Transductant colonies:				
Total	7658	4642	2231	1135
Tested	7113	4460	1826	930
Highly resistant	7053	4212	1	71
Slightly resistant	57	232	0	846
Sensitive: Total	3	16	1825	13
Wild type	3	4	not tested	0

sensitive, presumably wild type, and only one was resistant, presumably the result of a "reversion" in a recipient cell. In the experiments with *str-r* as donor, on the other hand, only a minute fraction of transductant colonies (three out of 7113 and four out of 4460) were wild type; the great majority were resistant, representing the donor type. Therefore, the results show conclusively that *str-d-77*, *str-r-2*, and *str-r-3* are closely linked.

The control data in Table 4 show the proportions of highly resistant, slightly resistant, and sensitive "revertants" when *str-d-77* bacteria were grown without transducing phage. It is evident that by far the largest number of the revertants (about 91%) were resistant to a low concentration of streptomycin (5 μg/ml) but not to a high concentration (1000 μg/ml); whereas a very few (about 1.4%) were sensitive to streptomycin, and the rest (about 7.6%) were resistant to a high concentration. In the experiments with *str-r-2* and *str-r-3*, most of the tested colonies were highly resistant to streptomycin; and, judging by the control data, a large majority of these represented transduction by the donor marker, only a small fraction being highly resistant "revertants" from *str-d-77*. All low-resistance types were presumably due to "reversions." Sensitive types could have originated either by "reversion" or by recombination between *str-d-77* and *str-r*. Sensitive "revertants" still carry the *str-d* gene, as demonstrated by results described later in this paper; and so it is possible to distinguish them from recombinants by transduction tests. Among the 19 sensitive colonies obtained in these experiments, seven were found to be wild type—and thus recombinants—whereas the other 12 were "revertants." So it is clear that recombination occurs between *str-d-77* bacteria and both *str-r-2* and *str-r-3*, but that the frequency of recombinants is very low—four in about 4000 in the case of *str-r-2* and three in about 7000 in the case of *str-r-3*.

Similar experiments were made with *str-d-5* as recipient and *str-r-2*, *str-r-3*, and *str-s* as donors. The results show conclusively that *str-d-5* is also very closely linked with the *str-r* markers. Recombinants were not obtained among 745 colonies tested in the experiments with *str-r-3* as donor, nor among 266 colonies in the *str-r-2* experiments.

Genetic analysis of str-d *"revertants":* The streptomycin-sensitive "revertants" from *str-d* were analyzed to determine whether they were due to reverse mutation at the same locus as the original change that gave rise to *str-d*, or to mutation at a suppressor locus, which modified the expression of the *str-d* mutant gene. If "revertants" were caused by reverse mutations, they could be expected to have the wild type genotype; whereas if they resulted from the action of a suppressor, they should still carry the original *str-d* gene and in addition have a new mutant gene at some other locus.

The genotype of the sensitive *str-d* "revertants" could readily be determined by transduction experiments in which they were the recipients and sensitive wild type bacteria were the donors. The infected cells were grown in medium containing a high concentration of streptomycin. In the event that the revertants had the true wild type genotype, only a few streptomycin-resistant colonies

could be expected—the result of mutations from sensitivity to resistance among the recipient bacteria. They should not exceed the number found on control plates containing sensitive "revertants" on streptomycin medium without transducing phage. On the other hand, if the "revertant" recipients carried the original *str-d* gene plus a mutation at a suppressor locus, all recombinations involving the suppressor locus would give rise to the *str-d* phenotype, and many streptomycin-resistant colonies having the properties of the original *str-d* mutant colonies would appear.

All together, 42 "revertants" were tested—30 obtained from *str-d-77* and 12 from *str-d-5*. Seventeen of them were sensitive to streptomycin and the other 25 were resistant to a low concentration. In every case, many *str-d* colonies appeared, showing conclusively that suppressor mutations had been responsible for the "revertants."

Structure of the suppressor locus: Experiments were made to determine whether the suppressor mutations involved one gene locus or more than one, and whether—in case only one was involved—recombination could be detected between mutants of independent origin. Reciprocal transduction tests were carried out with ten sensitive *str-d-77* suppressor mutants (*su-6-str*, *su-7-str*, *su-8-str*, *su-10-str*, *su-11-str*, *su-13-str*, *su-14-str*, *su-15-str*, *su-16-str*, *su-18-str*) and the wild type streptomycin-sensitive strain (Table 5). The data from the tests with wild type as recipient and suppressor (*su-str*) strains as donors provide information about the relative frequencies of transfer of the *str-d-77* marker by phage from the different strains. The data of the experiments with wild type as donor indicate the relative frequencies of transfer of the $(su\text{-}str)^+$ marker to the differ-

TABLE 5

Results of reciprocal transduction experiments between streptomycin-sensitive suppressor mutants (su-6-str, su-7-str, *etc.*) *derived from* str-d-77 *bacteria. (Number of streptomycin-dependent colonies found on plates containing 50 μg per ml of streptomycin)*

Recipient	Control	Donor										
		Wild type	*su-6*	*-7*	*-8*	*-10*	*-11*	*-13*	*-14*	*-15*	*-16*	*-18*
Wild type	2	1	161	326	177	161	406	490	113	139	65	243
su-6	0	80	0	3	0	0	1	0	0	0	0	3
-7	3	448	22	1	16	11	25	19	14	11	11	0
-8	0	137	1	2	0	0	3	0	0	0	0	8
-10	0	87	0	7	0	0	1	0	0	0	0	1
-11	0	182	2	12	1	1	0	3	1	0	2	14
-13	0	151	0	11	0	0	4	1	0	0	0	11
-14	0	126	0	12	0	0	0	0	0	2	0	6
-15	1	169	2	11	0	3	4	4	2	3	2	11
-16	0	52	0	5	0	1	0	0	0	0	1	7
-18	1	116	1	0	1	0	2	6	2	2	0	0

The data for wild type as recipient (top row) indicate the relative potencies of the donors in transduction of the *str-d-77* marker; and the data for wild type as donor (third column) indicate the relative potencies of the recipients in transduction of the *su-str* marker.

ent suppressor recipients. The "control" column indicates the number of spontaneous back mutations of the suppressor gene.

If two independent suppressor mutants represented mutations at two different gene loci, not closely linked, reciprocal transduction experiments between them would be expected to produce approximately the same numbers of dependent recombinants as would experiments between either one of them and a wild type donor. On the other hand, the numbers of recombinants would be smaller if the mutants were nonidentical alleles, or the result of changes at closely linked loci; and if they were identical alleles, no recombinants would be expected.

The data of the table show unmistakably that the ten suppressor mutants had originated through mutations either at one gene locus or at very closely linked loci. Since recombination between the different suppressor mutants either did not occur or was detected with very low frequency, the more likely possibility seems to be that one gene locus was involved and that the mutants were allelic. If this is so, the data demonstrate that recombination occurs between at least some alleles. No conclusions can be drawn, from these experiments, as to which combinations are unable to produce any recombinants, for the data are not adequate. Only three plates were used per experiment; and in addition the efficiency of transduction of the recipient bacteria was very low as indicated by the tests in which the wild type was donor. According to those tests, the bacterial culture of *su-7* showed the highest frequencies of transduction; and a large proportion of the tests of *su-7* with other donors produced detectible recombinants.

Two of these ten streptomycin-sensitive suppressor mutants were also tested with six "revertants" of *str-d-77* that were resistant to low concentrations of streptomycin (growing on about 5 μg per ml but not on 50 μg per ml). Results of the tests (Table 6) are similar to those obtained with the sensitive suppressor mutants. They indicate that all 16 of the tested mutants (Tables 5 and 6) carried alleles of the same suppressor locus. The larger numbers of recombinants obtained in the tests with the slightly resistant suppressor mutants were probably due to higher transduction efficiency of the recipient bacteria—indicated by their high frequencies of transduction with wild type donor.

TABLE 6

Results of transduction experiments between slightly resistant suppressor mutants (su-1, su-2, . . .) *derived from* str-d-77 *bacteria and two streptomycin-sensitive suppressor mutants*

Recipient	Control	Donor		
		Wild type	*su-7*	*su-11*
su-1	21	1030	116	51
-2	2	1086	96	3
-4	36	1570	108	281
-24	41	742	31	1
-28	55	785	39	58
-30	35	983	53	118

The third column indicates the relative potencies of the recipients in transduction of the *su-str* marker.

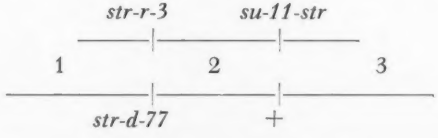
Linkage among str-d, str-r, *and* su-str *loci:* The experiments discussed so far showed that a mutation at a suppressor locus (*su-str*) was responsible for "revertants" among *str-d* bacteria, but they threw no light on the question of whether or not the *su-str* and *str-d* loci are close enough together to be carried in one transducing fragment. Experiments were designed especially to answer that question.

First a transduction test was made between recipient bacteria carrying both streptomycin dependence and a suppressor mutation, *str-d-77 su-11-str*, and donor bacteria carrying streptomycin resistance and no suppressor, *str-r-3* (*su-str*)+. Analysis of the colonies that appeared on transduction plates showed that three classes were represented: (1) resistant to a high concentration of streptomycin; (2) resistant to a low concentration of streptomycin; and (3) dependent on streptomycin. The genotypes of class (1) and class (2) colonies were determined by transduction tests with *str-d-77* (*su-str*)+ as recipient and bacterial cultures from individual colonies as donors. Eight class (1) colonies were tested, and all were *str-r* (*su-str*)+; whereas five class (2) colonies tested were all *str-r su-str*. Thus, in the transduction experiment

str-d-77 su-11-str (x) *str-r-3* (*su-str*)+

the donor genotype was recovered, *str-r* (*su-str*)+, as well as two recombinants, *str-r su-str* and *str-d* (*su-str*)+. Since a donor genotype representing two markers can be recovered only if both markers are carried in the same transducing fragment, these results show that the *str* and *su-str* loci are closely linked. They also reveal that *su-11-str* acts on *str-r-3* as well as on *str-d-77*. Bacteria of the genetic constitution *str-r-3 su-11-str* are resistant only to a low concentration of streptomycin.

In order to discover the approximate distance between the *str* and *su-str* loci, 420 colonies from an experiment *str-d-77* (*su-str*)+ (x) *str-r-3 su-str*, selected on medium containing no streptomycin, were picked at random, and their genotypes were determined by tests of their ability to grow on plates containing no streptomycin, 50 μg of streptomycin, or 1000 μg of streptomycin. Eighty-three of the colonies, or 19.7 percent, were *str-r su-str*+ (class A); 286, or 68.0 percent, were *str-r su-str* (class B); 50, or 11.9 percent, were *str-d su-str* (class C); and one was probably of mutational origin. This experiment can be illustrated by the following diagram:



The lower line represents the recipient bacterial chromosome and the upper line the transducing fragment. The numbers are inserted to designate the sections of chromosome lying between and beyond the loci concerned. Class A recombinants are produced by recombinations involving regions 1 and 2; class B

involve regions 1 and 3; and class C, regions 2 and 3. The fact that class B was considerably larger than either A or C indicates very close linkage between the *str* and *su-str* loci.

Specificity of su-str: Evidence already presented shows that *su-11-str* affects both *str-d-77* (dependence) and *str-r-3* (resistance). In addition, tests of *su-11-str* with *str-d-5* and *str-d-67* mutants indicated that these genes were affected in the same way as *str-d-77*. Therefore *su-11-str* is not allele specific.

It is interesting to note that *su-11-str* with either *str-d-5*, *str-d-67*, or *str-d-77* produced a streptomycin-sensitive phenotype, whereas with *str-r-3* it produced a phenotype resistant to low concentrations of streptomycin.

DISCUSSION

Results obtained in these transduction experiments with strain B/r of *E. coli* support a previous conclusion, based on the evidence of recombination experiments with strain K-12 (DEMEREC *et al.* 1949; NEWCOMBE and NYHOLM 1950), that resistance to high concentrations of streptomycin and dependence on streptomycin are controlled by alleles of the same gene locus. Genetic control of low, multistep resistance in *E. coli* has not yet been investigated, and consequently we do not know the relation between the gene governing one-step resistance and the gene or genes governing multistep resistance.

The fact that no recombinants were observed in transduction experiments with six streptomycin-dependent mutants indicates either that these *str-d* mutations occurred at very closely adjacent sites or, more probably, that they are multisite changes. It should be pointed out, however, that the technique for detecting recombinants in the streptomycin-resistance system is considerably less sensitive than the technique employed with auxotrophs.

Similar tests involving one *str-d* mutant as recipient and two *str-r* strains as donors produced very few recombinants and thus indicated a very close association also between *str-d* and *str-r* mutational sites.

It is well known that streptomycin-dependent bacteria may mutate to nondependent types, which are either sensitive or resistant to streptomycin. This mutability from dependence to nondependence has been put to extensive use in testing the comparative mutagenicity of various agents (DEMEREC 1951; DEMEREC, BERTANI, and FLINT 1951; DEMEREC and FLINT 1951). The recent development of a method for applying transduction techniques to *E. coli* made it possible to analyze the nondependent mutants genetically, and to determine whether the "reversions" are due to back mutation at the *str-d* locus or to changes in some other part of the genome. The results have been reported in this paper. They reveal that the "revertants" do not originate through true reversions at the *str-d* locus but are the consequence of independent mutations at another locus, which suppress the activity of *str-d*. These suppressor mutations are evidently not specific, but affect the expression of both *str-d* and *str-r* genes.

Sixteen suppressor mutants—all that were tested—proved to be allelic, an indication that only one gene locus is involved in suppression. Furthermore, it

appears that the suppressor locus and the locus governing resistance and dependence are close enough to each other to be transduced together.

Thus the results of these studies as a whole reveal that the genetic control of high streptomycin resistance and dependence in *E. coli* resides within a short region of chromosome, which also contains a genetic mechanism for control of the degree of resistance; and that reversions from dependence, although not due to changes in the *str-d* gene, are the result of mutations from the wild type at one single locus which is closely associated with the *str-d* locus.

SUMMARY

Genetic analyses have been made of streptomycin dependence (*str-d*), resistance (*str-r*), and sensitivity (*str-s*), as well as "reversion" from dependence, in *Escherichia coli* strain B/r by means of transduction experiments utilizing P1 phage. The results can be summarized as follows: (1) The *str-d* and *str-r* markers are transferable by transduction to *str-s* bacteria. (2) The *str-s* marker can be transferred to *str-d* bacteria. (3) In reciprocal experiments with six *str-d* mutants of independent origin, no recombinants appeared, an indication that all of them resulted from mutations at a single gene locus. (4) Experiments with two *str-d* mutants as recipients and two *str-r* mutants as donors indicated that *str-d* and *str-r* are nonidentical alleles, occupying very closely linked sites; for only a small number of streptomycin-sensitive recombinants appeared. (5) Analysis of 42 "revertants" derived from two *str-d* strains proved that all of them still carried the mutant *str-d* gene. In other words, mutation at another (suppressor) locus is responsible for "reversion" to nondependence. (6) Ten streptomycin-sensitive and six slightly resistant "revertants" were tested by reciprocal transduction for allelism of the suppressor mutations. The data indicate that all these *su-str* mutants resulted from an allelic series of changes at one locus. Several nonidentical alleles have been identified. (7) There is evidence of close linkage between the suppressor locus and the locus governing resistance and dependence. (8) There is evidence that the suppressor mutations are not allele specific. They affect the expression of both *str-d* and *str-r*.

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LEAF LOBATION IN GOSSYPIUM AMPHIDIPLOIDS AS DETERMINED BY LEAF-SHAPE GENES OF GOSSYPIUM DIPLOID SPECIES[1]

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INVESTIGATION of leaf lobation, or leaf-shape expression, is informative about phenotypic expression and is suggestive of evolutionary change in diploid and amphidiploid species of Gossypium. The problem at hand is the relationship of leaf-shape genes of the amphidiploid species and the leaf-shape genes of the closely related species of the A and D diploid genomes, since the Gossypium amphidiploids 2 (AD) are considered to have originated from a hybrid between a species similar to diploids of the A genome and a species similar to diploids of the D genome (BEASLEY 1940; HUTCHINSON, SILOW, and STEPHENS 1947; SKOVSTED 1933; STEPHENS 1944).

A developmental study of leaf lobation made by STEPHENS 1945c indicated that the common leaf shapes of the amphidiploid species are determined by the same genes as the leaf shapes of the diploid species of the A genome. This similarity of genes for leaf-shape expression is to be expected according to the hypothesis of HUTCHINSON *et al.* (1947), that the amphidiploid species have a recent common origin from an A genome species, presumable *G. arboreum* L. $2A_2$, crossed with D genome *G. raimondii* Ulbr. $2D_8$. It would appear then that the alleles at a leaf-shape locus of the amphidiploids are the same as the genes of the leaf-shape locus of the A_2 diploid.

Another range in leaf lobation is found among the various species of the D diploids, each phenotype presumably being controlled by genes at a single locus (STEPHENS 1945c). GREEN (1953), working with a new leaf shape in amphidiploid *G. hirsutum* L. $2A_hD_h$, assumed that the gene had been transferred from *G. thurberi* Tod. $2D_1$ via the synthetic amphidiploid $2A_2D_1$. A genetic test showed the new gene to be an allele of the gene for okra-leaf shape in the amphidiploid, and GREEN thus presumed the leaf-shape locus was located on the D genomes of the amphidiploids. RHYNE (1951) showed that a lobed-leaf expression of *G. thurberi* segregated in progeny of the synthetic hexaploid hybrid, *G. hirsutum* × *G. thurberi* $2A_hD_hD_1$, as expected if the leaf-shape locus were located on the D genomes of the hybrid.

An investigation using transferred leaf-shape genes of the A and D diploids in Gossypium amphidiploids is needed. The relationships of the genes of the diploids

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and the naturally occurring amphidiploids should fall in certain predictable patterns:

1. If the Gossypium amphidiploids are new, i.e., if they have a recent and common origin, the leaf-shape genes of the diploids may be shown to be the same as genes of the amphidiploids. The criteria for judging if they are the same are: The transferred leaf-shape genes of the diploids produce phenotypes that are identical with those of the amphidiploids. The transferred genes have the same dominance and epistatic gene interaction that the genes of amphidiploids have. And genetic segregation in appropriately marked hybrids shows that the transferred genes are located either at the same locus or at different loci. When the genes are located at the same locus, they can be called "identical" genes, but when they are located at different loci, and each locus is in a different genome of the amphidiploid, they can be called "duplicated" genes. A new amphidiploid generally is expected to have some duplicated genes.

2. If the Gossypium amphidiploids are old species and/or if they do not have a common origin, the leaf-shape genes of the diploids may be shown to be different from the genes of the amphidiploids. The criteria for judging if they differ are: The transferred leaf-shape genes of the diploids produce phenotypes that differ from those of the amphidiploids. The transferred genes and the amphidiploid genes have different dominance and epistatic gene interactions, and genetic segregation in appropriately marked hybrids shows that the transferred genes are located at the same locus or at different loci. When the genes are located at the same locus, they can be called "multiple alleles." Multiple allelism has been demonstrated for a number of transferred diploid genes at other loci of the natural amphidiploids (GREEN 1953; STEPHENS 1945a,b,c; HUTCHINSON *et al.* 1947; RHYNE 1951, 1958). When the genes are located at different loci and each locus is in a different genome of the amphidiploids, they can be called "independent" genes.

According to the HUTCHINSON *et al.* (1947) hypothesis of a recent and common origin it would appear that transferred genes for leaf lobing from the A genome diploids would behave in amphidiploids as identical genes of the alleles at the leaf-shape locus. It is possible, however, that a transferred gene from one D diploid may be identical with the common leaf-shape allele of one of the natural amphidiploids and may be allelic with the common allele of another amphidiploid. Such evidence for multiple allelism would be suggestive either of divergence among amphidiploids or possibly of multiple origin of the amphidiploid species.

This paper is concerned with genetic behavior of leaf-shape genes from various A and D diploids when transferred to Gossypium amphidiploid background. Emphasis is placed on genetic segregation and leaf-shape expression after the gene transference has been accomplished. Anomalous behavior of genes is, but Mendelian inheritance is not reported for the transference procedure. Gene relationships are to be determined from segregation ratios in progeny of appropri-

ately marked hybrids and from dominance and epistatic gene action in particular genetic backgrounds.

MATERIALS AND METHODS

Leaf-shape phenotypes were observed in the hot, arid Arizona and in the humid North Carolina climates. These expressions were essentially the same as those reported by STEPHENS (1945c) for the tropical Trinidad location. Consequently, the expressions figured by STEPHENS (1945c) have been partly adapted to construct Figure 1. Only the extremes in leaf lobation have been selected from the various expressions found in either the A or D diploids; but the intermediate expressions were found to conform to these reported patterns.

The selected alleles from the A diploids were laciniate and Asiatic "broad". Laciniate is the dominant allele of this multiple allelic series (HUTCHINSON

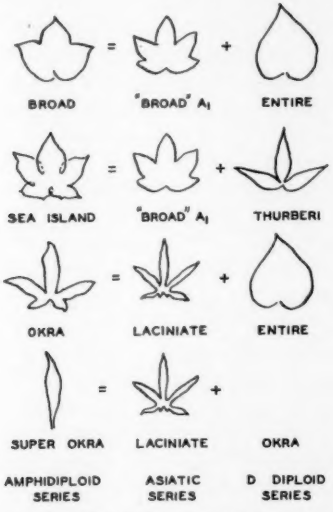

FIGURE 1.—Leaf lobation in amphidiploid and diploid Gossypium species. The column on the left contains the four common, leaf lobations and the alleles at a common locus of the natural amphidiploid species as they appear in Upland varieties of amphidiploid *G. hirsutum*. The center and right-hand columns show respectively the extremes of leaf-shape expression in the A and D diploid species. The amphidiploid phenotypes also could be produced by interaction of transferred, diploid, leaf-shape genes in the A and D genomes of the amphidiploids. For example, a broad leaf in the amphidiploids is the interaction product of Asiatic "broad" and D diploid entire genes, as is shown in the first row. Sea Island was the interaction of Asiatic "broad" and D diploid "thurberi", 2nd row. Okra-leaf shape was produced by Asiatic laciniate and D diploid entire, 3rd row. A super-okra phenotype could be produced by Asiatic laciniate and okra of the amphidiploid D genomes, 4th row. Okra-leaf shape is omitted from the right column of row 4 since it is not known in a D genome diploid. Okra and super okra are D genome alleles in the amphidiploids but they must be considered as evolution products of the amphidiploid species.

1934; STEPHENS 1945c) and Asiatic "broad" is the recessive. In Figure 1 both expressions are taken from *G. arboreum* $2A_2$, although the recessive form is commonly found in *G. herbaceum* L. $2A_1$. From the D diploids the extreme amount of leaf lobation is found in *G. thurberi* $2D_1$ which furnished the "thurberi" allele and the least amount, the entire (unlobed) leaf, is figured from *G. raimondii* $2D_5$.

Four alleles have been established in the natural amphidiploids (STEPHENS 1945a,c) ranging from a dominant strap-like leaf of super okra to a recessive broad-lobed expression. Any phenotype called broad refers hereafter to the recessive expression in the amphidiploids.

As a guide for the reader, Figure 1 shows the amphidiploid leaf shapes and the interactions of various diploid alleles that produce similar expressions in amphidiploid background. This procedure is necessary since a common amphidiploid phenotype will be shown to result from the interaction of more than one set of alleles. Figure 1 thus is a rearrangement of the figure and hypotheses of STEPHENS (1945c). The interaction of genes Asiatic "broad" and "entire" of the A and D diploid genomes, respectively, produces the broad-leaf expression of the amphidiploids. This conforms with the figure and hypothesis of STEPHENS. These expressions are found across the top row of Figure 1. The interaction of genes Asiatic "broad" and "thurberi" of the A and D diploid genomes, respectively, produces a lobed leaf. This phenotype is indistinguishable from the "Sea Island" expression in amphidiploid *G. barbadense* L. $2A_hD_h$. These are found in the second row from the top of Figure 1. The interaction of genes laciniate and recessive entire of the A and D diploid genomes, respectively, produces an okra expression in the amphidiploids. This conforms to the phenotype but not the location predicted by STEPHENS (1945c). These are found in the third row from the top of Figure 1. The super okra expression of the amphidiploids was produced by the interaction of genes laciniate and okra of the A and D genomes, respectively, of amphidiploid *G. hirsutum*. These are found in the bottom row of Figure 1. The phenotype for okra is missing from the D diploid series since the okra gene has never been identified in a D diploid species. Both okra and super okra may be considered to be evolutionary products of the amphidiploids.

For simplicity the extremes in phenotypic expression are reported herein. The laciniate and Asiatic "broad" alleles were transferred from the A_2 genome via new amphidiploids. The laciniate has been obtained from the A_2 of amphidiploids $2A_2B_1$ and $2A_2D_1$, and Asiatic "broad" from $2A_2D_1$. The transfer of these A_2 genes to the A genomes of the natural amphidiploids was feasible because of the high amount of chromosome homology in the A_2 and the A_h genomes in each hybrid with *G. hirsutum* $2A_hD_h$. Infertility was great in these hybrids.

The dominant gene for the "thurberi" lobation was transferred from the $2D_1$ genomes of $2A_2D_1$ to the D genomes of the natural amphidiploids. The recessive gene for entire leaf came from special hybrids. The entire-leaf genes were transferred from the D_5 and D_2 genomes to the trispecies hybrids, A_hA_2 D_hD_5 and $A_hA_2D_hD_2$, to the D_h genome of amphidiploid $2A_hD_h$. These gene transferences

were feasible because of the high degree of homology between chromosomes of the D diploid and the D_h genomes. An anomalous behavior is reported for the leaf-shape gene of $2D_1$ in its transfer to the D_h and D_b genomes.

After transference was accomplished the following hybrids were made, using leaf-shape genes of the natural amphidiploids in *G. hirsutum* and *G. barbadense* stocks. These stocks themselves often contained leaf-shape genes transferred from another amphidiploid. For example, super okra in B.(1) below was a backcross transfer from *G. hirsutum*, since super okra does not occur in *G. barbadense* (STEPHENS 1945b).

A. (1) Laciniate × super okra, *G. hirsutum* background. F_1 genotype $L^L/l\ l/L^S$
(2) Laciniate × "thurberi", *G. hirsutum* background. F_1 genotype $L^L/l\ l/L^T$

Both F_2 and backcross populations were grown. Randomly taken F_3 proof progenies were observed to confirm F_2 classification. The backcross parent was a stock of *G. hirsutum* having a broad-leaf expression and a recessive ($l\,l\ l\,l$) genotype.

B. (1) "Thurberi" × super okra, *G. barbadense* background F_1 genotype $l/l\ L^T/L^S$
(2) "Thurberi" × Sea Island, *G. barbadense* background. F_1 genotype $l/l\ L^T/L^E$
(3) "Thurberi" × okra, *G. hirsutum* background. F_1 genotype $l/l\ L^T/L^O$

Backcross populations were grown. F_2 proof progenies were observed. A broad-leaf stock was used in most cases, furnishing a recessive ($l\,l\ l\,l$) genotype. In a few instances Sea Island ($l\,l\ L^EL^E$) was used when super okra was involved.

Three methods were used to distinguish between leaf-shape phenotypes in a common population. The first two were visual approaches. The first approximation came from a climax leaf of the main stem taken 3–4 nodes above the first sympodium and 5–6 leaves below the terminal leaf. If two phenotypes appeared alike, as they often did in backcross A.(1), leaves were observed at nodes along the main stem below the sympodia. This presumed a difference in the time of initiation of leaf lobing, being a developmental pattern technique adapted from STEPHENS (1945a,b,c) and characteristic for certain diploid leaf-shape genes. The third method was to measure climax leaves and to calculate indices; each allele has a characteristic value (HUTCHINSON 1934; STEPHENS 1945b). In reference to the Sea Island leaf shape in Figure 1, the distance from the leaf pulvinus (the indention just above the "I" in Island) to the tip of the central lobe became L. The average distance from the pulvinus to the sinus of the central lobe, the point of the maximum cut in the leaf along the central lobe, was called S. The ratio S/L was taken from measurements of several leaves from several plants to determine dominance and gene interaction of alleles. The ratio W/L was obtained in a similar manner, being the width of the central lobe at its

widest place, usually at a place distal to the sinus of the leaf. The number of lobes was suggestive of particular leaf-shape genes, but this information was often inconclusive.

EXPERIMENTAL RESULTS

Genetic relationships as determined by segregation ratios

Duplicated phenotypes—duplicated genes

A deeply lobed leaf was observed in the amphidiploid $2A_2B_1$ and in its hybrids with recessive broad-leaf *G. hirsutum*. This phenotype, laciniate, was repeated in the backcross of the hybrid $A_2B_1 \times A_hD_h$ with broad-leaf stocks. A second phenotype typical of the recessive parent was present in the backcross in about the same frequency. Because of the infertility in this hybrid and in its backcross progeny, plants having laciniate in the backcross were again backcrossed with the broad-leaf stocks, and a ratio of one laciniate to one broad leaf was observed in the progeny. A third backcross repeated the one to one ratio. Laciniate plants in each backcross generation were inbred, and an F_2 segregation in a ratio of three laciniate to one broad-leaf plant was observed. The laciniate class in the F_2 populations was separated into a homozygous and a heterozygous component; separation by S/L calculated ratios was more accurate than visual classification, as judged from information from test crosses made to confirm a phenotype. The homozygous laciniate, $L^l\ L^l\ l\ l$, had S/L ratios from 0.07–0.10 and heterozygous laciniate, $L^l\ l\ l\ l$, had S/L ratios from 0.11–0.18. Similar data were obtained for laciniate from $2A_2D_1$.

A stock, now homozygous for laciniate and fully fertile, was crossed with a stock carrying super okra and the resulting hybrid (A–1) was inbred. The F_2 had 812 plants with deeply lobed leaves, S/L ratios from 0.00–0.18, and 67 plants with broad-lobed leaves, S/L ratios from 0.40–0.60. This segregation pattern has a P of 0.09 for an expected ratio of 15 dominant to one recessive. Classification in a 4:6:5:1 ratio was also made; the four had a super-okra phenotype, the six had a phenotype like that of the F_1 hybrid, and the five like heterozygous laciniate as described in the paragraph above. This 5/16 class composed one phenotypic class when mature leaves at flowering nodes were classified by measurement and visual methods; but progeny testing showed that 3/16 had been laciniate and 2/16 heterozygous super okra. The mature plant phenotype of the 5/16 class was produced by different genotypes, and a separation of genotypes was possible from juvenile plants. The first and second true leaves were much lobed in genotypes having a laciniate gene, while these leaves were essentially entire in genotypes having a super-okra gene.

The hybrid between laciniate and super okra was backcrossed to a broad-leaf stock. The duplicate factor ratio of three dominant to one recessive was observed; there were 56 deeply lobed to 23 broad-leaf plants. Both laciniate and super-okra heterozygous genotypes again produced a common phenotype. The doubly heterozygous genotype ($L^l\ l\ L^s\ l$) again produced the phenotype of the F_1 hybrid. The

largest S/L ratio value was 0.18 for any dominant phenotype, and the smallest value was 0.42 for the broad-leaf phenotype.

A homozygous laciniate stock was crossed with a stock having a lobed-leaf expression controlled by a dominant gene of $2D_1$ that had transferred ten backcross generations to *G. hirsutum*. The hybrid (A–2) was inbred and the F_2 population grown. A 15:1 ratio was expected since laciniate was an A_h genome gene and the "thurberi" a D_h genome gene. The F_2 showed 331 deeply lobed and 24 broad-leaf plants, and the backcross 76 deeply lobed to 24 broad-leaf plants. These observed values were in close agreement with the expected 15:1 F_2 and 3:1 backcross ratios. The various lobation phenotypes in the F_2 were divided into 12 "laciniate":3 thurberi:1 broad, and again each of the classes having the lobed leaves could be subdivided into homozygous and heterozygous genotypes. Interaction of leaf-shape genes changed the phenotypic expression when laciniate and thurberi genes were both present in a genotype. More about this interaction is given in the Gene action section.

Two contradictory locations of the amphidiploid leaf-shape locus were indicated earlier in the introduction. STEPHENS (1945c) suggested an A genome location; therefore laciniate being herein transferred to the A_h genome ought to be allelic with super okra. The observed ratio of 15 lobed leaf to one broad leaf, however, shows that laciniate and super okra are genes at independent loci; but this information does not place the super okra locus on the D_h genome. GREEN (1953) suggested the super okra locus was on the D_h genomes and, therefore, a 15 to one ratio is expected. He also suggested that *G. thurberi* $2D_1$ had contributed a new allele to the super okra locus, but he showed no evidence that the $2D_1$ contributed it. As expected in the hybrid laciniate and the leaf-shape gene from *G. thurberi* in a D_h genome location gave a 15 to one ratio. That "thurberi" leaf and super okra are alleles at a common D_h locus is shown below. It is germane here to note that GERSTEL and PHILLIPS (1958) reported segregation of okra and super okra-leaf shape in hexaploid hybrids $2A_hD_hD_5$. Their segregation in tetrasomic fashion involved loci of the D genomes of the hexaploid. STEPHENS (1955) subsequently reported the linkage of okra-leaf shape with crinkled of the D_h genome. The STEPHENS' hypothesis for genome location has been discarded.

Multiple allelic genes

A leaf-shape gene was transferred readily from the D_1 genome of amphidiploid $2A_2D_1$ to both *G. hirsutum* and *G. barbadense*. The Materials and Methods section indicates that interaction of the "broad" A_2 alleles and the D_1 genes produced a leaf shape similar to the Sea Island phenotype of *G. barbadense*. This expression is called "thurberi" leaf shape. In the cross of amphidiploid $2A_2D_1$ with broad leaf *G. hirsutum* the resulting hybrid plants were of two phenotypes. The thurberi leaf shape occurred in 300+ hybrid plants in a ratio of 19 to one for broad leaf. The exceptional broad-leaf hybrids also had all of the dominant genes of the A_2 genome that the thurberi-leaf hybrids had. In a cross of amphidiploid $2A_2D_1$ with *G. hirsutum* where the laciniate gene was used on the A_2 genome, GERSTEL and PHILLIPS (1957) indicated one plant only that did not have the laciniate ex-

pression; however, they considered the segregation between A and D genomes to be infinity to zero (no segregation). These two independent cases should be considered to be intergenomic A_2–D_1 segregation. BEASLEY (1940, 1942) reported occasional quadrivalents in his $2A_2D_1$ hybrid; genetic segregation would be expected from the particular A–D chromosomes involved in the anomalous quadrivalents.

Both the thurberi and the broad-leaf hybrids were crossed reciprocally with broad-leaf stocks of *G. hirsutum* The thurberi phenotypes segregated in a typical ratio of one thurberi to one broad-leaf pattern. One exceptional plant with an extra narrow leaf produced a good-fitting three thurberi to one broad ratio and was $L^T L^T ll$. The broad-leaf hybrids did not segregate nor did any of the randomly drawn broad-leaf plants of the test cross population. An example of typical segregation from a thurberi hybrid is a 1953 backcross population which was grown under summer (Arizona) conditions. There were 81 thurberi and 87 broad-leaf plants. Additional backcrossing repeated the one to one segregation ratio. The thurberi phenotype changed but little as the gene from D_1 was transferred to *G. hirsutum* D_h genomes by backcrossing.

Hybrids were made between the original $2A_2D_1$ amphidiploid and super okra and okra stocks; these crosses (B1,B3) are listed in the Materials and Methods section. The resulting hybrids were backcrossed with recessive stocks. The (B–1) hybrid having super okra and backcrossed to $L^E L^E ll$ produced 64 plants with a super okra heterozygous phenotype and 47 with thurberi and/or Sea Island, plus five broad-leaf plants. Randomly drawn plants from each phenotype were again backcrossed; the super okra heterozygotes produced only super okra and Sea Island plants in a typical 1:1 ratio. The thurberi or Sea Island phenotypes rarely segregated. The broad-leaf plants produced some but not all broad-leaf plants. The (B–3) hybrid having okra produced 306 deeply lobed and seven broad-leaf plants, and 127 of the 306 plants were classified as heterozygous okra and 179 as heterozygous thurberi. The phenotypes overlapped considerably in this interspecific backcross population. However, randomly drawn plants when testcrossed produced a one to one segregation from okra phenotypes and one to one from thurberi phenotypes. There was no error of classification. Subsequently after several backcrosses to broad-leaf stocks, plants having thurberi phenotypes were crossed with okra stocks, i.e., L^T/L^O hybrids were produced, and the F_2 segregation showed a good fit to a ratio of one thurberi; two F_1 phenotypes; one okra, and zero broad-leaf plants. Undoubtedly the thurberi leaf-shape gene was an allele of super okra and okra at a D_h genome location.

The occurrence of broad-leaf plants from the (B–1,B–3) hybrids should be considered to be intergenomic (A–D) segregation. MENZEL and BROWN (1954) diagrammed associations of chromosomes which indicated A-D pairing in hybrids similar to these. The replacement of a D_h or D_1 leaf-shape gene by the gene for broad leaf of A_2 would be detected as plants lacking a dominant leaf-shape gene, and 12 plants from (B-1, B-3) hybrids did not exhibit a dominant gene. Additional chromosomes besides those having leaf-shape genes could have been involved in

the A-D pairing as MENZEL and BROWN (1954) illustrated cytologically. Plants lacking allelic dominant genes for pollen color, corolla color, and petal spot of the A_2 and A_b or A_h genomes were detected. Dominant alleles of the D_1 and D_h genomes other than the leaf-shape genes were missing in the first backcross generation.

Genetic relationships as determined by gene action

Identical phenotypes = Controlled by either identical genes or similar alleles

Genetic tests showed no segregation for a number of genes of diploid species transferred to amphidiploid genomes. The data on gene action, as indicated by phenotype expression, dominance to known alleles, and interaction with leaf-shape genes at independent loci, were interpreted thusly: (a) The diploid gene was so similar to the amphidiploid leaf-shape gene in action that it was equivalent and probably identical with the amphidiploid gene at the particular locus, and (b) the diploid gene was similar to but not necessarily identical with an allele at a particular locus. Certain genes closely linked with the leaf-shape locus made identification of alleles at a locus uncertain. This stage (b) was reached by STEPHENS (1945a,b) when additional backcrossing did not refine the phenotypic expression of suspected "identical" alleles. The (b) stage also was reached in this study. Closely linked genes of known linkage groups from diploid species were shown to remain in parental combinations after many backcross generations (RHYNE 1958). In the absence of any means for breaking such close linkages, other than irradiation which can cause rearrangement, no effort is made here to separate the (a) and (b) patterns.

The gene for Asiatic "broad", the typical allele of *G. herbaceum* $2A_1$, was available herein in the $2A_2$ genome. When transferred to the A_h and A_b genomes, it did not segregate distinctly from the genes for the recessive broad-leaf phenotype. These A genome genes can be considered to be equivalents in gene action.

The gene for entire leaf, the recessive alleles of either *G. raimondii* $2D_5$ or *G. armourianum* $2D_2$, is equivalent to the allele at the D_h genome locus that is recessive to okra leaf shape.

The interaction of recessive Asiatic "broad" and recessive entire in amphidiploid background produces a broad-leaf phenotype. This result is in agreement with STEPHENS' hypothesis (1945c) that an interaction of recessive leaf-shape genes of the Asiatic A genomes with genes for entire leaf shape of $2D_5$ produces the broad-leaf expression of the amphidiploid species.

In the (B–1,2) hybrids the gene for thurberi leaf-shape did not segregate distinctly from the gene for typical Sea Island expression in *G. barbadense* background. A distinction could be made in late-flowering plants, where a thurberi expression was associated with late flowering and Sea Island with early flowering. Additional backcrossing failed to show that the leaf shape of the late-flowering plants was consistently thurberi; rather it appeared that in late-flowering background a Sea Island genotype would be modified into a thurberi expression. Attempts to free the leaf lobation from flowering influence were unsuccessful.

In *G. hirsutum* the thurberi expression was characteristic with a tendency for thurberi lobation to occur in late-flowering phenotypes. (It is worth while to remark that a Sea Island expression in *G. hirsutum* results from an introgressed gene of *G. barbadense*. Certain of the associated characters accompanying the *G. barbadense* gene are not removed by backcrossing.) The thurberi and Sea Island genes are of the (b) pattern.

Duplicated phenotypes—not necessarily determined by duplicated genes

Certain phenotypes of known leaf-shape genes of *G. hirsutum* were produced by various combinations of the laciniate gene from the A_2 genome in an A_h genome location. One level of laciniate, heterozygous at the A_h locus in an $L^L\ l\ l\ l$ genotype, produced a phenotype equivalent to that of heterozygous super okra, an $l\ l\ L^S\ l$ genotype. Two laciniate genes, homozygous in the A_h genomes in an $L^L\ L^L\ l\ l$ genotype, produced a phenotype equivalent to homozygous okra, an $l\ l\ L^O\ L^O$ genotype. (This was as STEPHENS 1945c predicted.) Two laciniate genes and two okra genes in an $L^L\ L^L\ L^O\ L^O$ genotype produced a phenotype equivalent to homozygous super okra, an $l\ l\ L^S\ L^S$ genotype. The potency of the L^S genes suggests that changes have occurred at the leaf-shape locus in the evolution of the amphidiploids.

Another way of showing similarity of phenotypes may be taken from data reported in Tables 1–3. In Table 1 the S/L ratios for two varieties of *G. hirsutum* are shown; e.g., the S/L ratio in one variety for heterozygous laciniate is 0.130.

TABLE 1

Phenotypic expression of laciniate genes in the A_h genome interacting with homozygous recessive genes of the D_h genome locus and dominance relationships of laciniate to broad

Genotype	Phenotypic expression observed	Dominance information			
		Expected value $(G_1+G_3)/2$	Deviation (expected minus observed)	Change $(G_1-G_3)/2$	Dominance value of L^L $d=$ (dev./change)
		S/L ratio values			
$G_1 = l\ l\ l\ l$	0.545a*				
(Broad)	0.447b				
$G_2 = L^L l\ l\ l$	0.172a	0.320a	0.148a	0.224a	0.66a
	0.130b	0.268b	0.138b	0.179b	0.77b
$G_3 = L^L L^L l\ l$	0.096a				
	0.089b				
		W/L ratio values			
$G_1 = l\ l\ l\ l$	0.559a				
	0.552b				
$G_2 = L^L l\ l\ l$	0.294a	0.386a	0.092a	0.172a	0.53a
	0.308b	0.424b	0.116b	0.128b	0.90b
$G_3 = L^L L^L l\ l$	0.214a				
	0.297b				

* The a and b represent different varietal backgrounds of *G. hirsutum* in different growing seasons.
** For explanation of S/L and W/L ratios see last section under Materials and Methods.

TABLE 2

Phenotypic expression of three alleles of the D_h genome interacting with homozygous recessive genes of the A_h genome locus and dominance relationships to broad

Genotype	Phenotypic expression observed	Dominance information: Expected value $(G_1+G_5)/2$	Deviation (expected minus observed)	Change $(G_1-G_5)/2$	Dominance value of alleles $d=$(dev./change)
	S/L ratio values				
$G_1 = l\ l\ l\ l$ (Broad)	0.545a*				
	0.447b				
	0.517c				
$G_4 = l\ l\ L^s\ l$	0.132a	0.279a	0.147a	0.266a	0.55a (super okra)
$l\ l\ L^t\ l$	0.261b	0.298b	0.037b	0.149b	0.25b (thurberi)
$l\ l\ L^o\ l$	0.184c	0.298c	0.114c	0.219c	0.52c (okra)
$G^5 = l\ l\ L^s\ L^s$	0.013a**				
$l\ l\ L^t\ L^t$	0.150b				
$l\ l\ L^o\ L^o$	0.079c				
	W/L ratio values				
$G_1 = l\ l\ l\ l$ (Broad)	0.559a				
	0.552b				
	0.541c				
$G_4 = l\ l\ L^s\ l$	0.293a	0.342a	0.049a	0.216a	0.22a (super okra)
$l\ l\ L^t\ l$	0.465b	0.492b	0.027b	0.060b	0.45b (thurberi)
$l\ l\ L^o\ l$	0.340c	0.363c	0.023c	0.178c	0.13c (okra)
$G_5 = l\ l\ L^s\ L^s$	0.126a				
$l\ l\ L^t\ L^t$	0.432b				
$l\ l\ L^o\ L^o$	0.155c				

* The a, b and c represent different varietal backgrounds of *G. hirsutum* in the same growing season.
** This value ordinarily has a 0.000 value, since there is a single lobe. Occasional leaves on an occasional plant have a small sinus of about 0.02, hence average was 0.013.

In Table 2 the S/L ratio for heterozygous super okra is 0.132 for the same varietal background. The W/L values of the two heterozygous genotypes is the same, about 0.290. Similarly homozygous laciniate had a value of 0.089 for the S/L ratio in Table 1 and homozygous okra had a value of 0.079 in Table 2. The two varieties are not the same, but the flowering responses are comparable. In each of these duplicated phenotypes the number of leaf lobes also was the same. There was no doubt that a common phenotype could be produced by genes of different genomes.

A pertinent question is whether a common phenotype is produced by the same gene when present in either one of the genomes of an amphidiploid. The question perhaps is moot since linkages of leaf-shape genes and adjacent loci have resisted recombination in backcross transference procedures. Even so certain evidence that a common phenotype is produced by different genes in each genome can be marshalled. This evidence depends on estimates of dominance and epistatic gene interaction. In Tables 1–3 dominance is taken to be the ratio(d), the deviation

TABLE 3

Phenotypes of two alleles of the A_h genome loci interacting with three alleles of the D_h genome loci

Alleles of the A_h genome	Phenotypes expressed as ratios: Alleles of the D_h genome locus					
	$L^s L^s$	$L^t L^t$	$L^s l$	$L^t l$	$l\ l$	$l\ l$
	S/L ratio values					
$l\ l$	0.013 G_5a	0.150 G_5b	0.132 G_4a	0.261 G_4b	0.545 G_1a	0.447 G_1b
$L^L l$	0.002 G_7a	0.056 G_7b	0.049 G_6a	0.142 G_6b	0.172 G_2a	0.130 G_2b
$L^L L^L$	0.000 G_9a	0.049 G_9b	0.069 G_8a	0.080 G_8b	0.096 G_3a	0.089 G_3b
	W/L ratio values					
$l\ l$	0.126 G_5a	0.432 G_5b	0.293 G_4a	0.465 G_4b	0.559 G_1a	0.552 G_1b
$L^L l$	0.100 G_7a	0.267 G_7b	0.142 G_6a	0.310 G_6b	0.294 G_2a	0.308 G_2b
$L^L L^L$	0.090 G_9a	0.181 G_9b	0.195 G_8a	0.303 G_8b	0.214 G_3a	0.297 G_3b

of the heterozygous genotype from the expected midpoint divided by the expected change in phenotype between the two homozygous genotypes. In Table 1, for example, the expected midpoint or G_2 (heterozygous laciniate) is ½ of $G_1 + G_3 = 0.320$. The observed G_2 value for the S/L ratio (variety background a) is 0.172. The observed midpoint deviates from the expected, or $0.320 - 0.172 = 0.148$ is the deviation. The dominance of laciniate makes the phenotype of G_2 more deeply cut than expected. The expected change, 0.224, in phenotype due to one laciniate gene in G_2 is ½ of $G_1 - G_3 = 0.224$ (the actual change resulting when $2L^L$ genes were substituted for l in the A_h genome). The dominance value of L^L is $d =$ deviation divided by change or $0.148/0.224 = 0.66$ for variety a. The complete dominance of laciniate to broad would have produced an actual change of 0.224 and a ratio of $0.224/0.224 = 1.00$. The heterozygous G_2 and homozygous G_3 genotypes also would have had the same phenotype and same measurement.

In Table 1 the dominance relations (d) of the laciniate gene are given for common varietal backgrounds, where laciniate is interacting with the genes for entire leaf of the D_h genomes. The laciniate gene is highly dominant for the S/L ratio in the heterozygous condition, as is indicated by the d value of about 0.70. In Table 2 the dominance relations of the super okra and okra genes interacting with A_h broad leaves are given. A value of about 0.50 fits the two D_h alleles. By assuming that the recessive genes of the A_h and the D_h are the same, i.e., the broad leaf of Asiatics and the entire leaf of D diploids are merely the expressions (STEPHENS 1945c) manifest in the two diploid backgrounds, and by further as-

suming that a dominant gene would produce the same lobing in a amphidiploid regardless of which genome carries the gene, the difference in the levels of dominance suggests that laciniate and okra are not the same genes. A more plausible speculation is that the recessive genes of the A_h are not the same as the recessive genes of the D_h genomes, therefore a difference in the level of dominance between the laciniate and the okra genes is expected.

The data of Table 2 suggest that thurberi leaf is not a true allele of the super okra series of genes. Its dominance is much less than that of either super okra or okra as calculated from the S/L ratio. In parental $2D_1$ (Figure 1) the leaf has a small sinus and an S/L ratio of 0.01–0.02. This ratio compares with 0.110–0.120 for laciniate of the *G. arboreum*. A transference of thurberi to the D_h genome produces a value of 0.150–0.180 for the S/L ratio (Table 2), while laciniate in the A_h genome shows 0.080–0.100 (Table 1). Furthermore, thurberi interacts with super okra and okra in heterozygotes: the S/L ratio is 0.00 for $L^T L^s$ and essentially 0.15 (near the thurberi value) in L^T/L^o heterozygotes. This suggests that okra is not dominant to thurberi but is to entire.

The information of Table 3 for two populations conforms to different quantitative inheritance models. The phenotypes of the nine genotypes expected in an F_2 do not support the duplicate-gene model. The genotypes having a super-okra gene and a laciniate gene produce a number of variations in phenotypes, not the single phenotype as is expected from duplicated dominant genes. There was interaction of genes in a manner somewhat similar to a model for an additive gene action in quantitative inheritance studies. The doubly heterozygous genotype $L^L l L^T l$ produces a phenotype that deviated from what might have been predicted on the basis of the homozygous parental genotypes. Variations like the latter, an epistatic model, were encountered when genes for lobed leaf (other than laciniate) from the A_2 genome are combined with the various D_h alleles in F_2 populations.

DISCUSSION

The leaf-shape locus of the natural amphidiploid species is placed on the D genomes (1) by the evidence that the gene for thurberi leaf, by transference, at a D_h locus is an allele of the super-okra series of genes and (2) by obtaining a duplicate factor ratio when the laciniate gene on the A_h genome is brought into an F_2 population with either the thurberi or the super-okra genes. This placement of the super-okra locus agrees with independently published evidence for a D genome location in the amphidiploid species.

In a new amphidiploid, as stated previously, the leaf-shape genes of the parental diploid genomes should be the same as leaf-shape genes of the amphidiploids. This expectation was confirmed for certain genes only of the A and D diploid genomes: (1) The genes which interact in amphidiploid background to produce a broad-leaf shape are the recessive genes for broad leaf of the A diploid genomes and the recessive genes for entire leaf of the D diploid genomes. (2) The genes which interact in amphidiploid background to give Sea Island expression are

equivalent to the recessive genes for broad leaf either of the diploid or amphidiploid A genomes and the gene for thurberi leaf of the $2D_1$ diploid species. Another way of producing the same phenotype also has been observed. A gene from the A_2 genome, the L^r allele, has been transferred to the A_h genome, producing a thurberi and/or Sea Island phenotype. Its genome location is its chief distinction from the Sea Island phenotype. STEPHENS (1945c) also suggested that lobing gene, L^s of A_2 would interact with an entire leaf and give the Sea Island expression. Even so, in the settling down of a new amphidiploid a transfer of genetic material from one genome to another is entirely possible. A transfer of thurberi to the A and recessive broad to the D genomes was shown for amphidiploid $2A_2D_1$ and its hybrids with *G. hirsutum* and *G. barbadense*. Sea Island might have been an A genome gene, but in the D genome it is stable as STEPHENS (1945c) reported for *G. barbadense*. It is not commonly found in *G. hirsutum*. It usually is found in types that have been introgressed with *G. barbadense*.

A number of phenotypes similar to normally occurring leaf shapes were produced by leaf-shape genes from the diploid genomes when transferred into amphidiploid background. Genes at independent loci were indicated by the F_2 segregation ratios, and the gene action studies suggested that the same gene probably was not duplicated at each locus. The laciniate and super-okra genes, each on a different genome, interacted and produced phenotypic expressions in F_2 that were typical of additive gene action in quantitative inheritance models. The laciniate and thurberi genes, also on different genomes, produced phenotypic expressions in F_2 typical of epistatic gene action in quantitative inheritance models. A duplication of a phenotype thus does not require the same genes or even identical gene actions. These diversifications sharply contrast with the duplicate dominant gene model often expected for an amphidiploid. The ability to produce a common phenotype by different gene actions is suggestive rather of an evolving amphidiploid; that is to say, diploidization is occurring in the amphidiploids. The gene action could suggest that the Gossypium amphidiploids are probably not recent in origin, and perhaps not even of a common origin.

Mutations of broad-leaf to okra and okra to superokra are recorded in the literature (STEPHENS 1945c). These three are the alleles of *G. hirsutum*, either from collections made in the wild or from cultivated forms. The *G. barbadense* collections show essentially but one allele, Sea Island, except for a rare occurrence of okra in var. *darwinii* STEPHENS (1945c). The equivalence of thurberi and Sea Island in *G. barbadense* background, the dissimilarity of gene action of *thurberi* (and presumably Sea Island) and the super okra and okra alleles, and the reduction of recombination in *G. hirsutum* linkages transferred to *G. barbadense*,[2] therefore collectively indicate divergence of *G. hirsutum* and *G. barbadense*. Alternatively these independently collected data may support a contention that the D genomes of *G. hirsutum* and *G. barbadense* could differ because each had

[2] Unpublished data of the writer, and DR. S. G. STEPHENS, Genetics Dept., N.C. State College, transferring. *G. hirsutum* to *G. barbadense* and *G. barbadense* linkages to *G. hirsutum*, respectively.

a different D diploid parent. (It is not possible at present to distinguish the two possibilities.) Evidence in support of divergence of D diploid and D amphidiploid genomes may be inferred from GERSTEL and PHILLIPS' 1958 report that the D diploid genomes, including the D_5 putative diploid parent of the D genomes HUTCHINSON *et al.* (1947), show less segregation than A diploid genomes in hexaploid hybrids involving *G. hirsutum* or *G. barbadense* and these diploid genomes.

SUMMARY

A transference of leaf-shape genes was made from the A and D Gossypium diploid species to the A and D genomes of natural Gossypium amphidiploids. The dominant genes from the A diploids gave duplicate factor inheritance with the dominant alleles of the amphidiploid occurring at the leaf-shape locus. The dominant genes of the D diploids exhibited allelism with the four alleles of the amphidiploid leaf-shape locus. The independence of the A diploid genes and the allelism of the D diploid genes collectively support a placement of the amphidiploid leaf-shape locus on the D genomes. The gene from *G. thurberi* $2D_1$ was commonly transferred to the D genomes of amphidiploids and infrequently to the A genomes. The transposition of this D diploid gene to each of the amphidiploid genomes perhaps illustrates a similar earlier transposition of an A genome gene during the settling down of a newly produced amphidiploid. The "thurberi" gene for leaf shape was shown to be similar to the Sea Island gene commonly found in *G. barbadense*. The entire leaf-shape gene from two different D diploids was similar and perhaps identical with the gene now present in the D genomes of broad-leaf *G. hirsutum* $2(A_hD_h)$.

A production of a common phenotype was possible from more than one combination of genes of the A and D diploids in amphidiploid background. Such diversification in gene action by the genes at each of the two amphidiploid loci was unexpected for the Gossypium amphidiploids, which sometimes have been assumed to be of recent and common origin. Rather this ability to produce a common phenotype with different genes of each genome of an amphidiploid suggests that Gossypium amphidiploids are evolutionary old species. Alternatively, the amphidiploids may not be old species but may have multiple origins at least of the D genomes. The gene interaction of the leaf-shape genes of each locus of amphidiploid *G. hirsutum* produced phenotypes that resembled models for epistatic gene action in quantitative inheritance studies. Neither the duplicate model nor the additive gene action model had a good fit to the observed interaction of leaf-shape genes in the amphidiploid.

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THE GENETICS OF FLOWERING RESPONSE IN COTTON. II. INHERITANCE OF FLOWERING RESPONSE IN A GOSSYPIUM BARBADENSE CROSS[1]

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IN the first paper of this series, LEWIS and RICHMOND (1957) called attention to the renewed scientific interest in the introduction of cotton stocks from tropical centers of variability and reviewed the collecting expeditions that have been made to Mexico and Central America in recent years. More than half of the collected stocks failed to set fruit in the temperate zone during the summer while all of them fruited readily in the greenhouse during the winter. The writers emphasized the fact that in the early development of the agricultural varieties of American Upland cotton, *Gossypium hirsutum*, in the Cotton Belt of the United States, only genotypes which were day-length neutral in fruiting habit persisted longer than the first growing season.

The literature on the physiology of flowering in plants is extensive. It was adequately reviewed by MURNEEK *et al.* (1948) and by LEOPOLD (1951) and by others they cited. Purely genetic studies are less extensive, but the genetic basis of flowering has been recognized since the classic work of GARNER and ALLARD (1920).

Although cultivated stocks of *G. hirsutum* account for almost all of the American cotton crop, cottons with staple lengths of over 1⅜ inches come from varieties of *G. barbadense*. Formerly Sea Island types were important in the Southeastern United States and the West Indies. Currently most American extra-long staple cotton is grown in the irrigated valleys of Arizona, New Mexico and the Trans-Pecos area of Texas. The primitive or semicultivated types of *G. barbadense* are of no less value in the improvement of commercial cottons than the primitive Uplands. Organized collecting expeditions have not been made to the center of variability of *G. barbadense* (the Andean valleys of Bolivia and Colombia) in recent years, but a few *G. barbadense* stocks from these regions have been obtained through colleagues in the United States and certain Latin American countries.

So far, all the *G. barbadense* stocks collected in the tropics exhibit a short-day reaction when grown under the long-day conditions of summer in the American

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Cotton Belt. One such stock PI 220040/CB 2933, was collected in 1954 by H. D. BARKER[3] in the region around Socorro, Department Santander, Colombia. The native name for this cotton was Lengupa, and it was used as the short-day parent in the experiment reported here. The day-neutral parent used in these studies was Pima S–1, the agricultural variety of *G. barbadense* grown in the Southwest. Pima S–1 has a complex genetic background and presumably has some *G. hirsutum* introgression in its pedigree.

PROCEDURE

The original cross between Pima S–1 and Lengupa was made under the direction of THOMAS KERR.[4] The parental F_1 stocks were grown at the tropical cotton garden in Iguala, Mexico, during the winter of 1955–56. Seeds were produced on the parents and the F_1 by controlled self-pollination. Also each parent was backcrossed to the F_1 to produce $F_1 \times$ Pima S–1 and $F_1 \times$ Lengupa seeds. The cotton genetics and breeding unit at College Station, Texas received the seed lots in March, 1956. Individual seeds were started in 6-ounce paper cups in the greenhouse. When approximately two weeks old the seedlings were transplanted to the genetics field nursery. Rows were 40 inches wide and the plants were spaced 24 inches in the rows. The plants were scored for "flowering" and "nonflowering" habit and the number of days from planting to first bloom was recorded for each flowering plant. Selfed seeds were obtained on plants which flowered, and appropriate nonflowering plants were transplanted to 10-inch pots in the greenhouse during the winter of 1956–57 in order to obtain seed for verifying the 1956 results with progeny tests in 1957.

As a precaution the plants were grown in a randomized and replicated design in 1956, but the data were pooled after a qualitative type of inheritance was indicated.

RESULTS

The flowering data for the 1956 planting of parental, F_1, F_2 and backcross material are recorded in Table 1. All plants of Lengupa, the F_1 Lengupa $\times$ Pima S-1 and the first backcross of Lengupa failed to flower or to initiate any visible fruit forms. The F_2 population of 147 plants segregated into two distinct classes in the ratio 3 nonflowering plants:1 flowering. The backcross to Pima S-1 gave a 1:1 nonflowering to flowering ratio. All the data indicated that flowering was controlled by one gene pair with the flowering recessive to the nonflowering habit.

On the hypothesis that the flowering habit in the material investigated in this experiment is controlled by a single recessive gene, the genotypes and correspond-

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ing phenotypes of the parent stocks and their hybrid progeny may be written as follows:

Lengupa	*F F*	Nonflowering
Pima S–1	*f f*	Flowering
F_1 Lengupa × Pima S–1	*F f*	Nonflowering
F_2 Lengupa × Pima S–1	1 *F F*	Nonflowering
	2 *F f*	Nonflowering
	1 *f f*	Flowering
F_1 × Lengupa	1 *F F*	Nonflowering
	1 *F f*	Nonflowering
F_1 × Pima S–1	1 *F f*	Nonflowering
	1 *f f*	Flowering

TABLE 1

Summary of data on flowering response of six progenies involving Lengupa and Pima S–1 grown during long days of summer, College Station, Texas, 1956

Progeny	No. plants		Chi-square	Mean no. of days to first flower*
	Flowering	Nonflowering		
Lengupa	0	29	—	No flowers
Pima S–1	28	0	—	72.6 ± 0.6
F_1 Lengupa × Pima S–1	0	27	—	No flowers
F_2 Lengupa × Pima S–1	42	105†	$\chi^2(1:3)=1.00$, p=.30–.50	75.6 ± 0.9
F_1 × Lengupa	0	82	—	No flowers
F_1 × Pima S–1	43	44	$\chi^2(1:1)=0.01$, p=.90–.95	74.1 ± 0.7

* Mean of plants which flowered.
† One anomalous late-flowering plant classified nonflowering.

The segregations obtained in the F_2 and the first backcross confirm this hypothesis.

In 1957 additional evidence of a critical nature was obtained from progeny analyses of randomly chosen plants from the backcross and F_2 populations scored in 1956. The results were recorded and analyzed by individual progeny, but the data from appropriate progenies were pooled on the basis of a homogeneous chi-square. The results, supported by data presented in Table 2, are summarized as follows:

Progeny tests of segregates from F_1 × Lengupa: All the plants in the backcross to Lengupa were nonflowering and, on the basis of the hypothesis, either *F f* or *F F* in genotype. Thirteen plants from this population were progeny tested. Segregation, in the ratio of 3 nonflowering:1 flowering, occurred in six of the progenies while no segregation for flowering habit occurred in the remaining seven. *Progeny tests of segregates from F_1 × Pima S–1:* The genotype of all the flowering plants should have been *f f*. All the plants in the progenies of four such plants flowered relatively early and profusely. Nonflowering plants in this population should have been heterozygous for the flowering gene, *F f* in genotype. Segrega-

TABLE 2

Summary of data of flowering response in progeny tests of segregates involving Lengupa and Pima S–1 grown during long days of summer, College Station, Texas, 1957

Progeny tests of segregates from:	No. of progeny	Assumed genotype	No. of plants flowering	Non-flowering	Chi-square (1:3)	Mean no. of days to first flower*
F_1 × Lengupa	6	*F f*	15	74	3.15 p=.05–.10	96.2 ± 1.6
F_1 × Lengupa	7	*F F*	0	103	—	No flowers
F_1 × Pima S–1	4	*f f*	72	0	—	88.2 ± 0.5
F_1 × Pima S–1	5	*F f*	32	110	0.46 p=.30–.50	88.6 ± 0.7
F_2 Lengupa × Pima S–1	6	*F f*	21	64	0.02 p=.50–.95	90.8 ± 0.9
F_2 Lengupa × Pima S–1	2	*F F*	0	29†	—	No flowers
F_2 Lengupa × Pima S–1	4	*f f*	33	0	—	89.3 ± 0.7

* Mean of plants which flowered.
† One anomalous late-flowering plant classified nonflowering.

tion occurred in the progenies of all five plants tested and on the basis of pooled data, segregation was in accordance with theoretical expectations, i.e., 3 nonflowering: 1 flowering.

Progeny tests of segregates from F_2 Lengupa × Pima S–1: As in the cases just summarized, the nonflowering plants in this population were assumed to be *F f* or *F F* in genotype while the genotype of the flowering plants was *f f*. Confirmation of these assumptions was obtained by scoring the F_3 progeny of 12 plants. Of eight nonflowering plants tested, six segregated in a 3 nonflowering: 1 flowering ratio in F_3 and, with one anomalous exception, none of the plants in the remaining two progenies segregated. On the anomalous plant, one flower occurred very late in the season. All the plants in the progenies of four flowering plants tested in F_3 flowered relatively early and profusely.

The mean number of days from planting to first flower of the flowering plants in each population is recorded in the last column of Tables 1 and 2. Comparisons of the mean differences among the populations were made by the *t* test, by the method of comparing groups of unequal size outlined by HAYES and IMMER (1942). In 1956 flowering plants from Pima S–1, F_1 × Pima S–1 and F_2 Lengupa × Pima S–1 flowered in 72.6, 74.1 and 75.6 days respectively. Pima S–1 was significantly earlier than the flowering F_2 segregates at the 0.05 level of significance, but there was no statistical significance in the other comparisons.

As recorded in Table 2, five populations contained flowering segregates in 1957. Comparisons among these means showed that the backcross of F_1 × Lengupa, which flowered in 96.2 days, was significantly later in flowering than the other four populations at the 0.01 level. Among these four populations the F_3 Lengupa × Pima S–1 segregates from nonflowering F_2 plants were slightly later than segregates in the backcross of F_1 × Pima S–1 regardless of whether the first backcross to Pima S–1 plants were flowering or nonflowering.

The 1957 plants flowered approximately two weeks later than the 1956 plants.

This seasonal difference was largely caused by a delay in transplanting in 1957. Ideally seedlings are transplanted when about two weeks old, but heavy rains at transplanting time in 1957 made it necessary to hold the plants in the greenhouse for approximately four weeks. Plants made little growth in the 6-ounce paper cups after two weeks.

DISCUSSION

The genetic study of the short-day, nonflowering response of Lengupa, a noncommercial stock of *Gossypium barbadense*, in a cross with Pima S–1, a day-neutral agricultural variety of the same species, showed that difference in flowering response in this material was controlled by one recessive gene pair.

Plants in this study either flowered profusely or not at all except for the anomalous behavior of two plants. In these two cases, only one flower occurred on each plant very late in the season. In vegetative characteristics the two plants resembled the nonflowering type. These cases of flowering of a "normally" nonflowering stock may be somewhat similar to the findings reported by WADDLE (1954), who studied photoperiodic response of short-day and day-neutral hybrids of American Upland stocks. Weak or sporadic flowering was observed in one of the short-day parental stocks which had not flowered in previous seasons. Further investigations showed that this stock rarely flowered at College Station, Texas, where the main experiments were conducted, but flowered moderately to abundantly at the U. S. Cotton Field Station, Shafter, California. Varying flowering responses were obtained at three other locations. Monofactorial inheritance of the flowering habit was indicated in one of the backcross populations, but there was no evidence of simple inheritance in the other hybrid populations. The experiments demonstrated that "the effects of critical short-day photoperiod can be modified by certain environmental elements other than length of day." WADDLE (1954) also stated that "the results obtained . . . all lend weight to the theory of a physiological system in which the genes controlling fruiting response in a given day length are operative only when other elements of the environment, particularly temperature, are interacting in such a manner as to permit their expression."

LEWIS and RICHMOND (1957) found even less evidence of simple inheritance in a cross of short-day *G. hirsutum* var. *marie-galante* and a day-neutral variety of American Upland cotton. In the first backcross populations the recurrent parent was dominant, and there was no segregation for flowering, i.e., backcrosses to the day-neutral parent all flowered while those to the short-day parent all failed to flower. The F_2 segregated for flowering and nonflowering habit, but the data suggested a quantitative rather than a qualitative mode of inheritance. While the data obtained in the study of two stocks of *G. barbadense* cotton reported here show clearly that flowering response is preponderantly under the control of a single gene pair, there can be little doubt that a more critical study under more controlled environmental conditions would have revealed the presence of modifying genes. In fact the writers, along with most other geneticists, hold that the same

can be said of all cases of so-called simple inheritance. The potency of the modifiers in the *barbadense* material must have been low, but they could have been present in sufficient numbers or potency in the two cases of flowering under discussion to account for the results obtained. At any rate the two plants in question were considered to be nonflowering in respect to the basic gene for flowering response.

Once flowering began, all the plants that flowered did so in a relatively short period. According to the hypothesis that flowering in the *barbadense* material used in this study occurred only in homozygous, *f f*, plants, the mean number of days from planting to first flower would be expected to be the same for flowering segregates in all populations. This was essentially the case although Lengupa and Pima S–1 appeared to differ in certain minor genes which affected time of flowering. These genes apparently accounted for the fact that flowering plants in progeny tests of segregates from the backcross of $F_1 \times$ Lengupa flowered later than those in other populations. By the same token their alleles also could have been responsible for the slightly earlier flowering of plants in the progeny tests of the backcross of $F_1 \times$ Pima S–1. Excepting progeny tests of the $F_1 \times$ Lengupa backcross plants, the greatest difference among other means in 1957 was 2.6 days. The statistical significance assigned to differences in mean flowering periods in two comparisons, other than those involving $F_1 \times$ Lengupa, obviously resulted from the remarkable uniformity in flowering period among the plants in the progenies in question.

The qualitative type of inheritance of flowering response of the *G. barbadense* stocks used in these experiments differed markedly from those obtained in earlier experiments involving stocks of *hirsutum*. In *barbadense*, short-day and day-neutral types were essentially under the control of one gene pair, the remainder of the genetic background having only a minor effect on flowering. However, in *hirsutum*, the complex inheritance patterns obtained in hybrid populations indicated that a number of rather potent genes must have been operating. According to HUTCHINSON, SILOW and STEPHENS (1947), both *barbadense* and *hirsutum* are natural amphidiploids and both originated in the New World. From the results of flowering response studied thus far in this series, it is obvious that the two species must have developed different genetic mechanisms for flowering during the course of their evolution.

SUMMARY

The inheritance of flowering response was studied in a cross between Lengupa and Pima S–1, short-day and day-neutral stocks, respectively, of *G. barbadense* cotton. Under natural long-day conditions of summer growth, difference in flowering response was controlled by one gene pair with the short-day, nonflowering response dominant to flowering.

The gene symbols *F* and *f* are proposed.

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A CYTOGENETIC STUDY OF CROSSING OVER IN INVERSION HETEROZYGOTES OF DROSOPHILA MELANOGASTER[1]

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THE recombinant offspring of *Drosophila melanogaster* females having free X chromosomes heterozygous for a long inversion are limited to even multiple crossover types. But from inversion heterozygotes in which the two X chromosomes are attached at their centromeric ends (= tandem metacentric compound X chromosome), single crossovers are frequently recovered. The synaptic configurations of these two structural heterozygotes are compared in Figure 1. Although this difference in recovery of single crossovers could be explained by supposing the products of single exchange in free X inversion heterozygotes to be inviable, there is no demonstrable lethality of zygotes produced by such females. The resolution of this apparent paradox was presented by STURTEVANT and BEADLE (1936) in their classic paper on the mechanics of crossing over in inversion heterozygotes; they proposed that single exchanges do occur in free X inversion heterozygotes, that the products of such exchange are invariably excluded from the functional egg nucleus, and that exclusion is accomplished by directed orientation of noncrossover chromatids towards the terminal products of the second meiotic division.

The cytological and genetic consequences of various kinds of exchange in free X inversion heterozygotes and in tandem metacentric compound X chromosomes

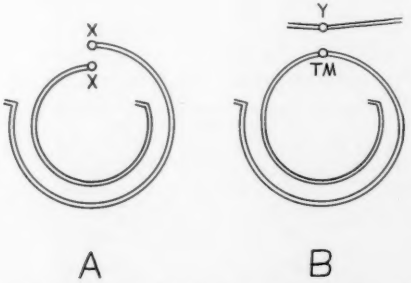

FIGURE 1.—Diagrammatic representations of the synaptic configurations of free X chromosomes heterozygous for a long inversion (A) and of the tandem metacentric chromosome (B). In the first meiotic division, two X centromeres will segregate in A whereas segregation of the Y and TM centromeres will occur in B.

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are summarized in Tables 1 and 2. The details of Table 1 have been supported by numerous cytological demonstrations of dicentric bridges and acentric fragments in a variety of plant and animal materials, but not in Drosophila; CARSON's (1946) investigation of inversion heterozygotes of the dipteran *Sciara impatiens* most closely approximates the expectations for Drosophila, although it is curious that no bridge free first divisions were observed in some 300 eggs examined. These cytological observations have not been accompanied by genetic data except in the notable work on maize inversion heterozygotes where breakage products of first division bridges are not always excluded from the ovules (RHOADES and DEMPSEY 1953). The genetic analysis of free X inversion heterozygotes and of the tandem metacentric compound X chromosome of Drosophila has been extensively advanced and has required only refinements of the original STURTEVANT and BEADLE model (NOVITSKI 1955). However, genetic studies on other types of compound X chromosomes, especially the reversed acrocentric and reversed ring

TABLE 1

Cytological and genetic results expected from crossing over in free X inversion heterozygotes (after STURTEVANT and BEADLE 1936)

Rank	Tetrad Type	Cytological results Divisions I and II*	Genetic results
E_0	..	normal	NCO
E_1	..	fragment + bridge	NCO
E_2	2s	normal	½ NCO + ½ DCO
	3sa	fragment + bridge	½ NCO + ½ DCO
	3sb	fragment + bridge	½ NCO + ½ DCO
	4s	2 fragments + double bridge	Nullo X

* The cytological results listed here are produced at division I but may persist in the first division plane during division II.

TABLE 2

Cytological and genetic results expected from crossing over in tandem metacentric compound X females (after NOVITSKI 1951 and 1955)

Rank	Tetrad Type	Cytological results Division I	Division II	Genetic results X-bearing gametes*
E_0	..	normal	normal	TM
E_1	a	fragment	fragment + bridge	lethal
	b	fragment	fragment	c X^c + (1—c) TM
E_2	2sa	normal	normal	½ TM + ½ CO TM
	2sb	normal	normal	TM
	3sa	fragment	fragment + bridge	lethal
	3sb	fragment	fragment	c X^c + (1—c) CO TM
	4sa	2 fragments	2 fragments	X^c
	4sb	2 fragments	2 fragments + double bridge	Nullo X

* TM = tandem metacentric, CO TM = tandem metacentric exhibiting homozygosis, X^c = single ring X chromosome, c = coefficient of non random disjunction.

(SANDLER 1958), have suggested a near or complete absence of single exchanges and a high frequency of zero and two exchange tetrads in these constructions. Consequently, the assumption that single exchanges occur freely within free X inversion heterozygotes is subject to question (SANDLER 1954).

In this report, cytological observations of bridges and fragments in the meiotic divisions of free X inversion heterozygotes and of tandem metacentric females of *Drosophila melanogaster* will be described, and the frequencies of these configurations will be compared with those expected from genetic data.

MATERIALS AND METHODS

The long inversion chosen for this study was *In(1)y*^{1} in which the inverted segment includes almost all the euchromatic portion of the X chromosome, or about 57 map units (for descriptions of this and other mutants, see BRIDGES and BREHME 1944). This inversion has been previously studied genetically (STURTEVANT and BEADLE 1936; NOVITSKI and SANDLER 1956) in both the free X and tandem metacentric combinations; in addition to these structural types, females homozygous for free X chromosomes of standard sequence were used in the present study. All females were heterozygous for various mutant markers and were three to five days old when producing eggs for either genetic or cytological data.

The eggs used for cytological preparations were obtained, one per female, by squeezing the egg from the uterus. Six to ten eggs were placed on an albuminized cover glass, covered with a drop of xylene for one minute to clear the chorion, punctured in the posterior region with a fine glass needle after removal of the xylene, and fixed by placing the cover glass in a Columbia dish containing Kahle's fixative. The fixed eggs were then prepared by the Feulgen whole mount procedure described by VON BORSTEL and LINDSLEY (1959). This method of egg collection provided a very poor yield of meiotic stages; a great majority of the eggs were in cleavage or later embryonic stages of development. In addition to the 100 eggs included in the data, there were 48 eggs in which no Feulgen positive material could be detected and 37 meiotic eggs rejected either because of being in stages inappropriate for classification for bridges and fragments or because of poor cytological quality. The male gamete was observed in transitional stages from elongated sperm head to rounded pronucleus in 90 of the 100 eggs recorded; no egg having more than one male gamete was seen. The eggs from the free X homozygotes and inversion heterozygotes were coded during cytological examination.

RESULTS AND DISCUSSION

The genetic results from all three types of females appear in Table 3. The data for the free X homozygotes and heterozygotes require little comment since they were quite typical of those ordinarily obtained from such crosses. In addition to the complete absence of single crossovers from the inversion heterozygotes, there was a reduced recovery of double crossovers. The frequency of double crossovers

TABLE 3

Genetic results of crosses of free X females homozygous for the standard sequence or heterozygous for In(1)y^4 *to* sc *males and of crosses of tandem metacentric females to* sc cv v f *males*

Type offspring	Parental female constitution		
	y/y^2 cv v f	*y^4/y^2 cv v f*	*v y^4 f w^m y/Y*
NCO	676 ♂ ♂	670 ♂ ♂	1799 TM ♀ ♀
SCO I	135 ♂ ♂	0	
SCO II	246 ♂ ♂	0	1528 X^c ♀ ♀
SCO III	179 ♂ ♂	0	
DCO I, II	8 ♂ ♂	6 ♂ ♂	
DCO I, III	39 ♂ ♂	12 ♂ ♂	52 CO TM ♀ ♀
DCO II, III	42 ♂ ♂	13 ♂ ♂	
Patroclinous	1 ♂	37 ♂ ♂	6749 ♂ ♂
Others	1269 ♀ ♀	749 ♀ ♀	15*

* Superfemales, detachment males, etc.

plus patroclinous males (produced from nullo X eggs) permit an estimate of 14.7 percent double exchange tetrads (E_2) in these free X inversion heterozygotes; no estimates of E_1 and E_0 are possible from these data.

The results from tandem metacentric females do allow estimates of E_0, E_1 and E_2 tetrad frequencies, although such estimates may be subject to considerable error. This is because the single ring (X^c) chromosomes generated by exchanges in this compound are deficient for the tip of the chromosome and duplicated for its base; such rings are inviable in the male and as $X^c/+$ females are only 76.9 percent as viable as sib M–5/+ females (NOVITSKI and SANDLER 1956). Using this observation as a viability correction factor, the 1528 X^c females observed (Table 3) were corrected to 2022 for further computations. (The senior author's choice of *In(1)y^4* for these experiments is most embarrassing because of the availability of a tandem metacentric chromosome especially constructed (NOVITSKI and LINDSLEY 1950) to avoid generated ring inviability). Using equations presented by NOVITSKI (1951), the following estimates were calculated from the tandem metacentric data: c = .70, E_0 = 13 percent, E_1 = 79 percent, and E_2 = eight percent.

It is perhaps gratuitous to describe meiosis in *Drosophila melanogaster* females as less than favorable cytological material; the figures are extremely small, the "large" chromosomes are seldom distinguishable, the "small" chromosome IV is seldom detectable, and the entire process subsequent to fertilization at Metaphase I is apparently rapid. The second meiotic division spindles are arranged in tandem near the anterodorsal surface of the egg, and the terminal product of meiosis nearest the center of the egg becomes the functional egg nucleus. Our limited observations of meiosis in standard sequence free X homozygotes were in accord with previous descriptions (SONNENBLICK 1950).

Reference to Table 1 shows that in free X inversion heterozygotes single and three strand double exchanges lead to the formation of a dicentric bridge and an

acentric fragment at the first meiotic division; these structures are illustrated in Figures 2A and 2B. In a few cases, the bridge extended from pole to pole, but in most cases the bridge was situated either midway between the poles or in one or the other half of the figure. In 16 of 17 division I figures having a bridge, a small acentric fragment was also observed, usually midway between the poles and near, but not in, the longitudinal axis. The coiled structure of the bridge and the remoteness of the fragment in Figure 2B were atypical. Some, if not all, of the bridges formed in the first division persisted during the second division (Figures 2C and 2D); in these cases, the bridges, and the fragments if present, were located

FIGURE 2.—Drawings (ca. 1975 ×) of meiotic figures from free X inversion heterozygotes (A-D) and from tandem metacentric females (E-H). A, B, E, and F are division I figures and C, D, G and H are division II figures. The figures drawn were selected as those most nearly parallel to the microscopic field, and in all cases, the lower end of the figure was directed towards the center of the egg.

in the plane of the first division spindle, indicating their exclusion from the egg nucleus.

Single and three strand double exchanges in the tandem metacentric chromosome cause fragment production in the first meiotic division (Table 2), but there is no accompanying bridge since the X and Y centromeres segregate in the first division; this cytological expectation is illustrated in Figures 2E and 2F. No cases of first division bridges were found in the tandem metacentric material. Among the single and double exchanges which cause fragment production in the first division, only half are expected to cause second division bridges, assuming no strand preference in exchange participation. Nine second division figures were observed with fragments in the first division plane, and among these, five exhibited bridges in one of the two second division planes (Figure 2G), two revealed no bridges in either second division plane (Figure 2H), and two others could not be classified for bridge presence or absence in one of their second division planes. It is intended that the fate of these second division bridges will be described in a separate report.

Four strand double exchanges are expected to generate two acentric fragments in the first division; these fragments should be accompanied by double bridges in division I of free X heterozygotes and in division II of tandem metacentrics. No configurations of this sort were observed, but this fact is not significant for if the frequency of E_2 tetrads is taken to be 14.7 percent and if one fourth of these are four strand doubles, then only three figures of this type were expected among the 85 figures observed.

The simplest approach to a quantitative treatment of the cytological results is to consider the frequencies of normal meiotic figures, that is, those figures which had neither bridges nor fragments. Since the frequency of normal figures equals $E_0 + \frac{1}{4} E_2$ in all cases (Tables 1 and 2), direct comparisons as well as sums can be made (Table 4). In both the free X inversion heterozygote and the tandem metacentric data, there were large differences in normal figure frequencies between the first and second divisions; however, these differences were not statistically significant and, being of opposite sign, cancelled each other when totalled for both types of chromosomes. Thus the data revealed no real differences in normal figure frequency between the first and second divisions or between the two kinds of chromosomes; furthermore, they indicate that $E_0 + \frac{1}{4} E_2 = 34$ percent. This estimate may be compared with that calculated from the tandem metacentric genetic results where $E_0 + \frac{1}{4} E_2 = 15$ percent.

We do not believe the difference between the cytological and genetic estimates of $E_0 + \frac{1}{4} E_2$ to be significant because of probable sources of error which may have caused either the cytological estimate to be too high, the genetic estimate to be too low, or both. The classification of a meiotic figure as normal depended upon failure to observe either an acentric fragment or a dicentric bridge. The fragments were so small as to be difficult to detect if removed from the vicinity of the meiotic figure; in the free X inversion heterozygote material, there were three cases in which a bridge, but not the expected fragment, was observed. This source of error

TABLE 4

Cytological results observed in meiotic figures in eggs from free X females homozygous for the standard (+) sequence or heterozygous for **In(1)y^4** *and from tandem metacentric (TM) females*

Female constitution	Meiotic division	Number of eggs			
		Total	Normal*	With fragment	With bridge
+/+	I	8	8	0	0
	II	7	7	0	0
	I + II	15	15	0	0
In/+	I	21	4 (.19)	16	17
	II	9	5 (.56)	2	4
	I + II	30	9 (.30)	18	21
TM/Y	I	44	18 (.41)	26	0
	II	11	2 (.18)	9	5 (+2?)
	I + II	55	20 (.36)	35	..
In/+ and TM/Y	I	65	22 (.34)		
	II	20	7 (.35)		
	I + II	85	29 (.34)		

* Numbers in parentheses are frequencies of normal with respect to total eggs.

may have been prominent in the first division tandem metacentric material where detection of an exchange depended solely upon observing the acentric fragment. It is also possible that broken bridges, if sufficiently retracted towards the poles, would have escaped cytological detection. Earlier reference has been made to the dependence of the tetrad rank frequencies inferred from the genetic data upon a correction for X^c female inviability. Although there is no question but that such a correction is appropriate, the actual amount of the correction is necessarily arbitrary. If the number of X^c females had been adjusted by a factor of 1.5 rather than 1.323, then the estimates of E_0, E_1 and E_2 would have been 21, 69 and ten percent, respectively, and the value of $E_0 + \frac{1}{4} E_2 = 23.5$ percent; however, the value of $c = 0.88$ on this basis is suspiciously high. In view of these considerations, it is satisfying that the genetic and cytological results agree as well as they do.

SUMMARY

Genetic and cytological data were collected from eggs produced by *Drosophila melanogaster* females of three structural types with regard to their X chromosomes, namely, free X standard sequence homozygotes, free X inversion heterozygotes and tandem metacentric compound X females; the inversion used was *In(1)y^4* which involves almost all the euchromatin of the X chromosome and which composes one of the arms of the tandem metacentric chromosome.

The cytological observations were in qualitative agreement with those predicted by previous genetic analyses: bridge and fragment configurations were observed

in the first division plane of free X inversion heterozygotes, while in the tandem metacentric eggs, fragments appeared in the first division plane and bridges appeared only in the second division plane. Considered quantitatively, the cytological results from free X inversion heterozygotes and tandem metacentrics were equivalent. The incidence of meiotic figures having neither bridges nor fragments and representing $E_0 + \frac{1}{4} E_2$ tetrads was 34 percent; this value and that of 15 percent estimated from genetic data were judged to be compatible when probable sources of genetic and cytological error were considered.

ACKNOWLEDGMENTS

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CHROMOSOMAL POLYMORPHISM IN DROSOPHILA PARAMELANICA PATTERSON

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RECENT work carried out chiefly with Drosophila has revealed a wealth of chromosomal and genetic variability in natural populations, and in addition has begun to indicate some of the evolutionary relationships which exist between species, as well as the complex genetic systems which are responsible for the maintenance of the intraspecific chromosomal and genetic polymorphism.

The *Drosophila melanica* species group appears to offer unusual opportunities for such studies. The members of the group are truly "wild", and unlike some domestic Drosophila species, are relatively little affected by man's activities. They occupy a broad geographical range, and the different species will in many instances cross to form fertile hybrids. Finally, at least some members of the group show a high degree of chromosomal polymorphism.

For a number of years the author has been engaged in a long-term study of the interspecific chromosomal changes which have occurred in the evolution of this group, and as one of the necessary preliminary steps, a study of the intraspecific variation has been undertaken.

Previously the only species in the group which had been thoroughly studied in regard to its chromosomal polymorphism was *D. melanica melanica*. In this species WARD (1952) studied salivary gland chromosomes derived from 65 laboratory strains, and found that the X chromosome showed four inversions differing from Standard, chromosome 4 showed two inversions and chromosome 2 showed 16. No inverted sequences were found in chromosomes 3 or 5. WARD also found that there were some differences in geographical distributions of the various gene sequences. It is the purpose of the present paper to report a series of cytological surveys carried out on *Drosophila melanica paramelanica* Patterson.

PATTERSON (1943) split the species *D. melanica* Sturtevant into the two subspecies *D. m. melanica* and *D. m. paramelanica* on the basis of rather minor differences in morphology, different geographical distributions and a rather high degree of reproductive isolation in the laboratory. It is now known that the males of these two forms show completely distinct differences in genitalia, and in addition there are differences in the egg filaments. It is the opinion of the author, that in the light of the total known differences the two forms constitute distinct species, and they will be so treated in this paper.

In the northeastern part of the United States five members of the *D. melanica* group are encountered; they are: *D. melanica* Sturtevant, *D. paramelanica* Patter-

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son, *D. melanura* Miller, *D. nigromelanica* Patterson and Wheeler and an undescribed species from Nebraska, Iowa, Missouri, Illinois, Indiana, Kentucky, Tennessee and Georgia, "species X".

D. nigromelanica is readily distinguished from the other four species on the basis of adult morphology and color. The remaining four species are easy to confuse in females, but may be separated on the basis of the male genitalia (see MILLER 1944 for diagrams).

D. paramelanica will form hybrids of both sexes with *D. melanica*, *D. melanura*, *D. nigromelanica*, *D. euronotus* (a species apparently confined to the southeastern United States) and "species X" (GRIFFEN 1942; MILLER 1944; PATTERSON and WARD 1952; STALKER, unpublished). GRIFFEN reports that all hybrids obtained in the crosses between *D. paramelanica* and the two species *D. melanica* and *D. nigromelanica* were fertile in both sexes. The author found these hybrids to be male sterile. Hybrids of *D. paramelanica* × *D. melanura* and *D. paramelanica* × *"species X"* are fertile in females but sterile in males.

D. paramelanica has been collected in Nebraska, Iowa, Minnesota, Missouri, Wisconsin, Michigan, Illinois, Indiana, Ohio, Tennessee, Pennsylvania, Maryland, New York, Maine, Vermont, Connecticut, Massachusetts and Southern Ontario. This predominantly northern species shows a broad zone of overlap with the southern *D. melanica:* this zone runs approximately through Nebraska, Missouri, Illinois and eastwards to lower New York.

Ecologically relatively little is known about *D. paramelanica*. CARSON and STALKER (1951) found flies breeding on the slime fluxes of infected Red Oak, Black Locust and Chinese Elm in the St. Louis area, which were identified as "*D. melanica*". Since St. Louis is in the zone of overlap, it is uncertain whether the specimens were *D. melanica*, *D. paramelanica* or "*species X*". It is the author's experience that *D. paramelanica* is most readily collected in low-lying deciduous forests, especially if they contain Oak, Elm or Willow. Stands of Birch in northern Michigan seemed to be free of the species. As in many other Drosophila, collecting is especially favorable near water.

D. paramelanica does not build up large populations in nature early in the year, and even as far south as St. Louis, trapping is difficult before early July. Wild populations in Missouri remain in breeding condition until late fall, and there is no evidence that any of the autumn females go into a reproductive diapause of the sort reported in *D. robusta* (CARSON and STALKER 1948).

MATERIALS AND METHODS

The material used in this investigation was for the most part derived from fresh collections made by the author and others. Some use was made of stocks which had been maintained in the laboratory for a number of years, especially where these were from areas for which fresh material was unavailable.

Wild-caught or stock flies from various areas were mated to one of two laboratory strains, both of which were homozygous for the common "Standard" gene arrangements. The chromosomes of the F_1 larvae were then examined for inver-

sion loops, using acetic-orcein squashes. Various techniques were used to derive the maximum information from the rather limited wild material which could be obtained. Wild-caught males were analyzed by examination of up to seven F_1 larvae, including at least one female, thus indicating the arrangements in their X chromosomes and autosomes. Wild-caught females, if virgin, were analyzed by the same technique, except that up to seven female larvae were examined. Wild-caught inseminated females were allowed to use up their sperm, and then mated to the laboratory Standard stock: when they did not survive the elimination of their sperm one of their sons was analyzed, and treated as if he were a wild-caught fly. Comparison of the wild-caught males, and the F_1 sons of wild-caught females captured at the same time in a large population in Iowa has shown no difference in inversion frequencies between the two classes, so that this treatment is probably justified. In the analysis of any fly, when seven smears of F_1 larvae failed to show more than one chromosomal sequence in either the X or chromosome 2, the fly was considered to be homozygous for the particular chromosome concerned. In many cases examination of fewer than seven F_1 larvae was needed to establish the structural heterozygosity of the wild parent.

All flies were reared on standard cornmeal-karo-agar-tegosept food, seeded with live yeast. Larvae to be used for salivary gland smears were fed a thick live yeast suspension during the latter part of their development to insure large, cytologically favorable salivary gland chromosomes. All rearing was carried out at 25 ± 1.0°C. Adults were frequently stored at 17°C until they could be used.

RESULTS

The chromosomes of D. paramelanica

In females the oogonial and neuroblast metaphase chromosome complement consists of five pairs of chromosomes: a long pair of approximately equal-armed V-shaped X chromosomes, a shorter pair of V-shaped autosomes (corresponding to salivary gland chromosome 4), a long pair of rods, a somewhat shorter pair of rods, and a pair of very short rods or dots. On the basis of length alone one might suppose that the longer pair of rods corresponds to salivary gland chromosome 2, and the somewhat shorter pair to salivary gland chromosome 3, although this is uncertain.

In male neuroblast cells the Y is a large equal-armed V-shaped chromosome, which in all strains studied by the author is easily distinguished from the X chromosome by the fact that with acetic-orcein it stains more heavily than any of the other chromosomes.

In salivary gland preparations each cell contains seven euchromatic arms, one of them very short. Chiefly on the basis of their tips, the euchromatic arms are easily homologized to those of *D. melanica*, and the author has followed WARD's system of nomenclature, applying the same number and left-right designation to the homologs in *D. paramelanica*. Thus the seven salivary gland arms are designated: XL, XR, 4L, 4R, 2, 3 and 5. In many instances XL·XR and 4L·4R

associations are clearly indicated when the X or 4 breaks free of the chromocenter but retains its interarm attachment.

STURTEVANT and NOVITSKI (1941) have shown that the chromosome arms found in various species of Drosophila may be homologized by comparison of mutant genes found on them. They divide the Drosophila chromosomal material into six "elements", A, B, C, D, E and F. In *D. melanogaster* element A is the rod-shaped X chromosome, B and C are the two arms of Autosome II, D and E the two arms of Autosome III, and the F the dot-shaped Autosome IV. Although it now seems clear that there may have been opportunities for gene exchange between the elements in the course of evolution, it has been demonstrated that in many cases groups of genes associated in a single element in one species are so associated in other species; in other words the breakdown of the six elements has not been extensive. A number of species of Drosophila with V-shaped X chromosomes represent fusion between the regularly sex-linked element A and one of the other elements which is autosomal in *D. melanogaster*. For example in the *D. obscura* group of species, the two-armed X represents a fusion of elements A and D. In the discussion of that species group the authors point out that the "sex-ratio" genotype is regularly associated with the D element of the X chromosome. They also mention that the "sex-ratio" trait is known in *D. melanica*, and that this would lead to the suggestion that the X of *D. melanica* likewise represents fusion of elements A and D. The present author has not been able to demonstrate "sex-ratio" in the species referred to in this paper as *D. melanica*, and the above reference presumably refers to *D. paramelanica*. If we follow STURTEVANT and NOVITSKI's suggestion that the two-armed X of the *D. melanica* species group represents an A-D fusion, then we can go further and designate arm XR as element A, since it carries the sex-linked recessive gene singed near its distal tip. The only other known mutant likely to be confused with singed is forked, which is also on element A of *D. melanogaster*. If the designation of XR as element A is correct, then we might expect to find the genotype associated with the sex-ratio trait on XL, or element D. Preliminary studies indicate clearly that the sex-ratio genotype in *D. paramelanica* is associated with XR. This leads to the rather surprising preliminary conclusion that in this species the sex-ratio genotype is associated with element A, rather than with element D as in the *D. obscura* group. However, as indicated by STALKER (1958) the sex-ratio genotype in *D. paramelanica* is of two types, distinguished by responsiveness to Y chromosome suppression, so it is entirely possible that both XR and XL carry sex-ratio genotypes. If it can be demonstrated that arm XL does *not* carry the sex-ratio genotype, then one might reasonably assume that there had been an interelement exchange in the evolution of either the *D. melanica* group or the *D. obscura* group. This problem is being studied further.

The standard chromosome map

In Figure 1 are shown the relative lengths of the seven euchromatic arms. In this figure all distances are given as percentages of the total length of the longest

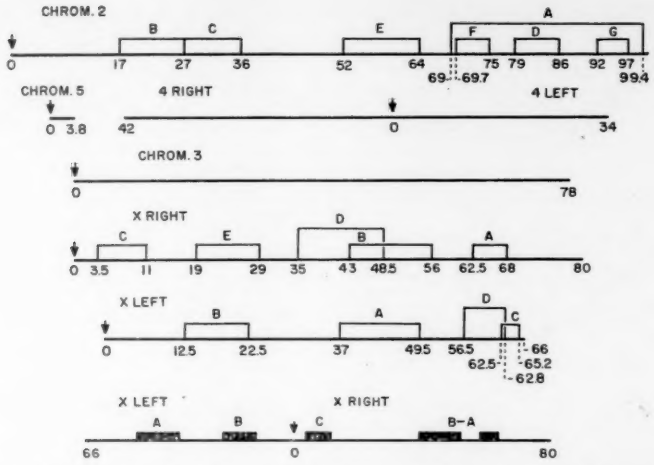

FIGURE 1.—Relative lengths of the euchromatic arms of the salivary gland chromosomes of *D. paramelanica*. In this figure all distances are given as percentages of the total length of the longest single arm, chromosome 2. Locations of known inversions are shown by brackets, and the centromere regions are indicated by arrows. At the bottom of the figure the two arms of the X chromosome are redrawn on a smaller scale to indicate the relative positions of the five sex-linked inversions showing adaptive association.

single arm, chromosome 2. The map distances were obtained from measurements of large numbers of photographs of acetic-orcein squashes of the homozygous Standard stocks: 28–12 and PR944a. In each arm, the position of the centromere, or more precisely the proximal limit of the euchromatin, is indicated by an arrow. The positions of those sections which may be inverted relative to Standard are indicated by brackets, and inversions themselves are given the symbols: A, B, C, etc. within each chromosome arm. In this paper no attempt has been made to homologize the system of inversions in *D. paramelanica* with those described in *D. melanica* by the use of corresponding inversion symbols; in fact it appears that few if any of the inversion differences in one species are homologous to those in the other. At the bottom of Figure 1 the two arms of the X chromosome are drawn on a smaller scale to emphasize the relative positions of the five important inversions which are to be discussed later in relation to the X chromosome inversion associations.

Figure 2 consists of photographs of the chromosome arms, showing the Standard sequence in all cases. Here as in Figure 1, the proximal ends of the chromosome arms are indicated by arrows, and the same numbering scale is used in both sets of illustrations. The limits of some of the inversions indicated in Figure 2 must be taken as approximate, and some may be in error by as much as two or three single bands.

In good preparations all of the major chromosome arms may be recognized

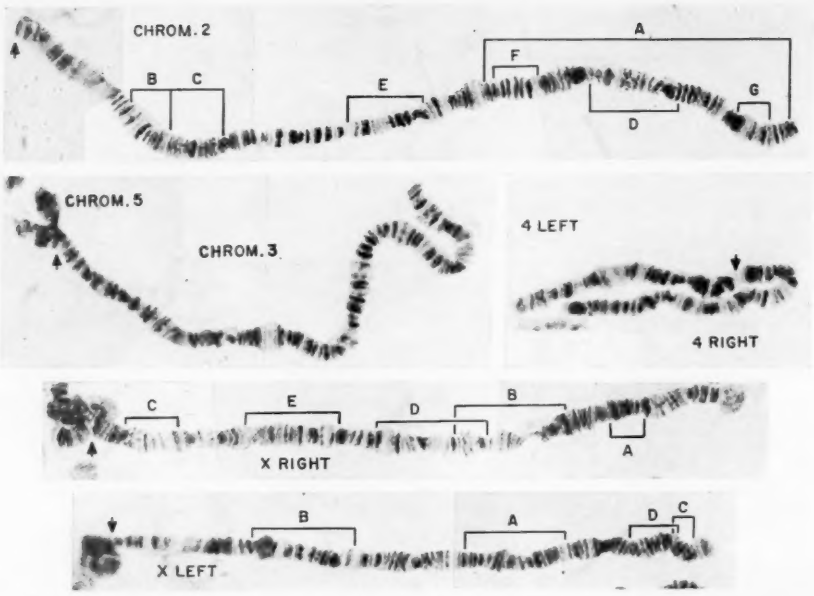

FIGURE 2.—Composite photographs of the salivary chromosome arms of *D. paramelanica*, showing the Standard banding sequence. The proximal ends of the arms are indicated by arrows, and the numbering system is the same as that used in Figure 1. The lettered brackets indicate the positions of the known inversions.

readily by the banding pattern at the tips. In poor preparations the tip of XL is extremely variable, and where difficulty is found in identifying this arm by the tip, the series of puffs found along its length constitute useful auxiliary characteristics.

Chromosome arm XR regularly shows a swelling at the tip, and very frequently exhibits a weak spot which may become stretched out and break, located approximately in the middle of inversion XR B.

Chromosome 2 has a weak spot at the base which is located approximately eight bands proximal to the proximal limit of inversion 2 C. The chromosome often breaks at this point.

In a few cells of some preparations chromosome arm 4L may show peculiar median adhesions resulting in figures superficially resembling inversion loops.

Geographical variability in chromosome 2

The known geographical ranges of the 16 gene sequences of chromosome 2 are indicated in Table 1. In examining the data for possible geographical trends in frequencies, it should be kept in mind that the second chromosome may be divided into two quite distinct regions, the proximal B-C region and distal A-F-D-G

region. That crossing over occurs between these two in nature is not surprising, and is proven by the finding of the reciprocal types such as AB, AC, A, B, C and + (here the symbol + indicates the Standard sequence for the pertinent inversions). The relationship of the rare median inversion E is unclear; it has been found only three times (in northern Michigan). Within the B-C region only the three types B, C and + are known, and no crossing over is expected between B and C. Within the A region, the three short inversions F, D and G which are included would not be expected to cross over with A, and there is no evidence that they have ever done so.

Considering first the proximal B-C region and ignoring for the moment the median and distal regions, the frequencies of the three classes B, C and + may be extracted from Table 1. If the various collecting sites are grouped into three broad zones, Northern, Middle and Southern, then it is clear (see Table 2) that arrangement C is northern, B is predominantly southern, and that + has its lowest frequency in the south.

The second section of Table 2 indicates the general clinal tendencies exhibited by the distal section of chromosome 2. Here the two commonest sequences, + and A replace each other, with A common in the north and nearly absent in the south and + the reverse. As expected, because of the association with A, sequences such as AG, AF, AD and ADG are commonest in the north.

Due to the recombination possible between the proximal and distal series of gene sequences, the phylogenies for the two should be considered separately. The basal +–B–C region could be represented phylogenetically as B⟷+⟷C, and since all three types are common in one region or another, and all but C widespread, it is difficult to guess which sequence was ancestral, although it seems probable that it was not C because of its restricted and northern distribution.

In the tip of chromosome 2 the phylogenies which can be established are illustrated in Chart 1. In this case the step from A to ADG could go through either AD or AG, as indicated by the alternate pathways. Both the + and A sequences are commoner and more widespread than the other four. This would suggest that either + or A is ancestral, and of the two perhaps + would be the likeliest candidate as it is typically southern while A is a northern sequence.

Since both the proximal and distal gene sequences in chromosome 2 show geographical frequency differences, it is not surprising that the proximal and distal ends show an interrelationship, such that the northern second chromosomes are characteristically + or C proximally and A distally (+ A or C A), while the southern chromosomes are B proximally and + distally (B +). Of somewhat more interest is the fact that even *within* a given population the two ends may not be associated at random. This is clearly shown in the sample from Mt. Vernon,

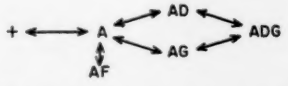

CHART 1.—Phylogeny of gene sequences in the tip of chromosome 2.

TABLE 1. *Geographical variability in gene arrangements of chromosome 2*

Locality	N	Standard	A	AC	AB	AD	ACF	ABD	ABE	ACD	ACE	ADG	AG	AE	B	C	ACDG
St. Louis, Mo.	44	6	..	..	..	..	..	..	..	..	..	..	..	..	38	..	..
		13.6	..	..	..	..	..	..	..	..	..	..	..	..	*86.4*	..	..
Watseka, Ill.	10	6	1	..	..	..	..	..	..	..	..	..	..	..	3	..	..
Terre Haute, Ind.	..	*60.0*	*10.0*	..	..	..	..	..	..	..	..	..	..	..	*30.0*	..	..
Tennessee: Sevierville,	10	..	..	..	..	..	..	..	..	..	..	..	..	..	10	..	..
Gatlinburg	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*100.0*	..	..
Iowa City, Iowa	23	10	3	.	..	..	..	..	..	..	..	..	..	..	9	1	..
Mason City, Iowa	..	*43.5*	*13.0*	..	..	..	..	..	..	..	..	..	..	..	*39.1*	*4.4*	..
Mt. Vernon, Iowa	251	140	17	19	..	..	..	..	..	..	..	..	1	..	66	8	..
	..	*55.8*	*6.8*	*7.6*	..	..	..	..	..	..	..	..	*0.4*	..	*26.3*	*3.2*	..
Plainwell, Mich.	36	11	11	6	2	2	..	..	..	1	..	..	..	..	1	2	..
	..	*30.6*	*30.6*	*16.7*	*5.6*	*5.6*	..	..	..	*2.8*	..	..	..	..	*2.8*	*5.6*	..
Little Falls, Minn.	95	3	38	39	11	2	1	1	..	..	..	..	..	..	..	..	..
	..	*3.2*	*40.0*	*41.1*	*11.6*	*2.1*	*1.1*	*1.1*	..	..	..	..	..	..	..	..	..
Lincoln, Humbolt,	16	6	2	..	1	..	..	..	..	..	..	..	..	..	7	..	..
Nebraska	..	*37.5*	*12.5*	..	*6.3*	..	..	..	..	..	..	..	..	..	*43.8*	..	..
Iron River, Wis.	8	..	4	3	..	1	..	..	..	..	..	..	..	..	..	..	..
	..	..	*50.0*	*37.5*	..	*12.5*	..	..	..	..	..	..	..	..	..	..	..
Iron Mt., Mich.	37	..	30	3	2	..	..	..	..	..	1	..	..	1	..	..	..
	..	..	*81.1*	*8.1*	*5.4*	..	..	..	..	..	*2.7*	..	..	*2.7*	..	..	..
Petoskey, Mich.	13	..	10	1	..	1	..	..	1	..	..	..	..	..	..	..	..
	..	..	*76.9*	*7.7*	..	*7.7*	..	..	*7.7*	..	..	..	..	..	..	..	..
Cold Spring Hbr., N.Y.	28	14	2	..	3	..	..	..	..	..	..	..	..	..	9	..	..
	..	*50.0*	*7.1*	..	*10.7*	..	..	..	..	..	..	..	..	..	*32.1*	..	..
Millersburg, Penn.	†	..	..	..	..	..	..	..	..	..	..	*	..	..	*	..	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Guarette, Maine	†	*	..	..	*	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
South Hero, Vt.	†	..	..	*	..	..	..	..	..	..	..	..	..	..	..	*	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Bridgewater, Vt.	49	12	18	6	..	4	..	..	..	1	..	1	..	..	..	3	4
	..	*24.5*	*36.7*	*12.2*	..	*8.2*	..	..	..	*2.0*	..	*2.0*	..	..	..	*6.1*	*8.2*
Total chromosomes	620	208	136	77	19	10	1	1	1	2	1	1	1	1	143	14	4
Total localities	16	10	11	8	6	5	1	1	1	2	1	1	1	1	9	5	1

In the body of the table the actual numbers of chromosomes of each kind from a given locality are shown by the upper figure; the lower italicized figure gives the percentage frequency.
* Indicates the presence of a given gene sequence.
† In the left column of the table shows that one or more laboratory stocks were the source of the chromosomes examined.

TABLE 2

Frequencies of selected gene sequences in chromosomes 2 and X

Zone	Chromosome 2							X chromosome	
	Proximal			Distal					AB·CBA CAB·CBA
	+	B	C	+	A	AG-AF	AD-ADG	+·+	AB·CB
North: Minn., Wis.,	125	15	62	18	168	1	15	109	11
N. Mich., Vermont	*61.9*	*7.4*	*30.7*	*8.9*	*83.2*	*0.5*	*7.4*	*90.8*	*9.2*
Middle: Iowa, N.Y.,	219	98	37	286	64	1	3	144	37
S. Mich., Nebraska	*61.9*	*27.7*	*10.5*	*80.8*	*18.1*	*0.3*	*0.8*	*79.6*	*20.4*
South: Mo., Ill.,	13	51	0	63	1	0	0	17	11
Indiana, Tennessee	*20.3*	*79.7*	*0*	*98.4*	*1.6*	*0*	*0*	*60.7*	*39.3*

In chromosome 2 the proximal and distal inversions are treated separately. In the X chromosome both arms are listed, in the order: X-Left·X-Right. For each cell in the table the upper figure indicates the number of chromosomes studied, the lower italicized figure the percentage within the particular geographical zone.

Iowa (see Table 3). In this table the computations of the expected numbers of proximal-distal combinations are based on the inversion frequencies within the population and the assumption of random association between the two ends of the chromosome.

From the left-hand section of Table 3 it will be noted that the agreement with expectation is not close: the χ^2 value of 81.0 leads to a P value of less than 0.001. In the right-hand section of the table the typically northern and typically southern associations, and the recombinant associations are lumped for a single comparison with their expected frequencies. Here again the discrepancy between observed and expected appears to be significant, and indicates that there is a tendency for northern and southern associations to persist even in intermediate populations where both northern and southern gene sequences occur with high frequencies.

Although other populations do not show significant non randomness of association in the second chromosome, the smaller sample sizes and relative lack of heterogeneity in the two ends of the chromosome make the detection of such non randomness unlikely in these populations.

GRIFFEN (1942) in discussing a preliminary cytological study made of *D. paramelanica*, states that using a Madison, Wis. strain as a Standard (he does not state that the strain was structurally homozygous, and the context suggests that it was not) he made a cross with a Wooster, Ohio strain and the F_1 showed two short overlapping inversions at the base of the long autosome (apparently chromosome 2). The position of these inversions certainly suggests 2-B and 2-C, and the most reasonable explanation of GRIFFEN's observation seems to be that his Madison Standard carried one inversion, the Wooster strain the other, so that he was observing a 2-A/2-B heterozygote. If this is the correct explanation, then the inversions 2-B and 2-C should perhaps be shown as overlapping. In the course of the present work 2-B/2-C heterozygotes have not been observed, and since comparisons of each of them with the Standard indicate they are adjacent rather than overlapping, they are shown in the former relationship. In addition to the two short inversions in the base of "the longest autosome" GRIFFEN reports that

Madison and Wooster strains differed by "two small inversions in another autosome". Another autosome would mean chromosome 3 or chromosome 4, and thus GRIFFEN's report would indicate heterozygosity in one of these autosomes. The material on which the present report is based does not include strains from Ohio, and the author has found no inversion heterozygosity in either chromosome 3 or 4 from other areas.

The X chromosome

In the discussion of variability in the X chromosome which follows, the two arms will be treated together. In all listings of gene sequences the sequence in the left arm (XL) will be given first: thus +·CBA represents a chromosome carrying the Standard sequence in the left arm and inverted sequences C, B and A in the right. The raised dot represents the centromere region.

The 13 types of X chromosomes found in wild populations are listed and their frequencies given in Table 4. This table does not include Standard (+·+) chromosomes from the following: Nebraska (2); Mason City, Iowa (4); Terre Haute, Ind. (1); Watseka, Ill. (4); Lincoln, Neb. stocks (2); Guarette, Me. stock (1); Hero, Vt. stock (1); Millersburg, Pa. stock (1).

A fourteenth type of X chromosome not listed in Table 4 has been found in an old laboratory stock from Cold Spring Harbor, N.Y. This chromosome, which may have been derived by crossing over in the laboratory, is B·CB (i.e., carries inversion B in XL and C and B in XR).

It is clear from examination of Table 4 that the commonest types of X chromosomes are: the Standard sequence +·+, and the *family* of sequences that include the inversions AB·CB (AB·CBA, CAB·CBA, AB·CB). Except for the three rare types DAB·CBA, +·D and +·E, all other chromosomes observed may be derived from Standard and the AB·CB family by crossing over.

Considering for the moment only +·+ and the AB·CB family, it is clear (see Table 2) that +·+ is relatively more frequent in the north, while the AB·CB family has higher frequencies in the south. It is worth noting here that in all cases tested (42 to date), the AB·CB family of chromosomes have been shown to carry the "sex-ratio" gene or gene-complex. Males with a "sex-ratio" X chromosome may under certain circumstances produce primarily X-bearing sperm and thus, daughters, but very few sons (STALKER 1958). Similar genes are known in the *D. obscura* group of Drosophila, and in this group as in *D. paramelanica*, X chromosomes carrying the sex-ratio gene have a characteristic gene sequence not found in normal X chromosomes. Likewise in these species it has been found that the frequency of the sex-ratio chromosomes is higher in the south than in the north (see WALLACE 1948).

With the exception of D and B in the right arm, there are no overlapping inversions known in the X chromosome. However, certain inversions are found only in association with others: thus A in XR has not been found unless associated with the adjacent inversion B, although XR B may be found without XR A. This suggests that crossing over may not occur between them and that their relationship is of phylogenetic origin. In XL the small distal inversion C has been found

TABLE 3

Chromosome 2 associations of proximal and distal gene sequences from a population in Mt. Vernon, Iowa

	Typically northern + A	Typically northern C A	Typically southern B +	Recombinants + +	Recombinants B A	Recombinants C +	Total northern-southern	Total recombinants
Observed	17	19	66	140	0	8	102	148
Expected	22.6	3.9	56.5	134.4	9.5	23.1	83	167
Difference	−5.6	+15.1	+9.5	+5.6	−9.5	−15.1	+19	−19
	$\chi^2 = 81.0$						$\chi^2 = 6.5$	
	$P = <0.001$						$P = 0.01$	

In the table the sequence at the proximal end is listed first, the distal last. Thus, chromosome type: + A has the standard proximal sequence and the inversion A distally. The expected frequencies are computed on the basis of the frequencies of the proximal and distal gene sequences within the Mt. Vernon sample, and on the assumption of random association between sequences in the two ends of the chromosome.

TABLE 4

Geographical variation in frequencies of X chromosomes from wild populations

Chromosome type XL · XR	St. Louis, Mo. N	%	Iowa City, Iowa N	%	Mt. Vernon, Iowa N	%	Little Falls, Minn. N	%	Iron River, Wisc. N	%	Iron Mt., Mich. N	%	Petoskey, Mich. N	%	Plainwell, Mich. N	%	C.S.H., N.Y. N	%	Bridgewater, Vermont N	%
+ · +	12	46	6	86	117	75	53	76	2	50	17	81	11	79	5	63	12	63	26	96
AB · CBA	11	42	1	14	26	17	7	10	..	..	1	5	..	..	1	7	..	..	..	..
CAB · CBA	..	..	..	..	5	3	2	3	..	..	2	10	..	..	..	..	..	..	..	..
DAB · CBA	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
+ · CBA	1	4	..	..	3	2	..	..	..	..	..	..	..	..	1	7	..	..	..	..
AB · CB	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	21	1	4
+ · BA	..	..	..	..	1	1	2	3	..	..	..	..	..	..	..	..	1	5	..	..
AB · C	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
+ · C	1	4	..	..	..	..	..	..	..	..	..	..	..	..	1	7	1	5	..	..
A · +	..	..	..	..	..	..	1	1	..	..	1	5	..	..	..	..	..	..	..	..
AB · +	1	4	..	..	..	..	..	..	1	25	..	..	2	25	..	..	1	5	..	..
+ · D	..	..	..	..	1	1	5	7	1	25	..	..	1	13	..	..	..	..	..	..
+ · E	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Totals:	26	..	7	..	156	..	70	..	4	..	21	..	8	..	14	..	19	..	27	..

This table does not include + · + chromosomes from the following: Nebraska (2); Mason City, Ia. (4); Indiana (1); Illinois (4); and from the following laboratory stocks: Lincoln, Neb. (2); Guarette, Me. (1); Hero, Vt. (1); Pennsylvania (1).

nine times in three different localities, but always in the association CAB CBA. In this case XL C and XL A appear to be sufficiently separated so that crossing over between them might be expected to occur, and the probable explanation of the absence of other combinations of XL C is that such combinations are genetically nonadaptive and are eliminated as they are produced by crossing over in the +·+/CAB·CBA females.

Associations within the AB·CB family of X chromosomes

In order to clarify the section to follow, a slight change in symbols will be employed. The + symbol will be used to represent the Standard sequence for *each* pertinent inversion, rather than the Standard sequence for the whole arm. Thus a female heterozygous Standard/AB·CBA will be represented as + +·+ ++/A B·C BA.

In certain sections of both XL and XR proof exists that crossing over may occur in nature between adjacent inversions in heterozygous individuals. This proof is based on the finding of all four reciprocal classes involving any two adjacent sections of chromosomes. For example, the existence in natural populations of the four chromosome types: + +·+ ++, A B·C BA, + +·+ BA and A B·C ++ indicates that crossing over may occur between XR C and XR BA. Similar evidence of crossing over exists between XL A and XL B and between XL B and XR C.

In order to obtain crossover X chromosomes for laboratory study, females with one Standard X chromosome, and the other X carrying either the inversions A B·C BA or A B·C B+ were prepared. The system of inversions in chromosome 2 of these females was not controlled; some were homozygous for the short basal second chromosome inversion 2-B, some were heterozygous 2-B/ Standard. The females were all heterozygous for two or more of the sex-linked recessive mutants: singed, garnet, carnation and brown, which were well distributed over the two arms. By picking out sons of the heterozygous females which showed recombination of the mutant genes, and subsequently analyzing their chromosomes, a number of crossovers were obtained. The approximate rates of crossing over for each region are given below, with the figures in parentheses indicating the number of classified individuals leading to each value: XL-A — XL-B = 0.06 percent (4,872); XL-B — XR-C = 0.10 percent (4,184); XR-C — XR-BA = 0.09 percent (9,915). There is no evidence that crossing over occurs between XR-B and XR-A, and thus they are treated as a single segment, XR-BA. On the basis of the data above it is possible to divide the X chromosome into the four separable segments: A B·C BA.

If in a given population, the indicated segments may be separated by crossing over, then the population might be expected to show random associations of them with the frequency of each type of association determined by the population frequency of the given segments. Any significant departure from random association between the various chromosome segments might be accounted for by drift, natural selection, or by recent phylogenetic origin of certain associations and insufficient time for their breakdown by crossing over. In the data to be ana-

lyzed below it will be shown that the four segments of the X chromosome are not randomly associated within populations and that this cannot be attributed to drift, since the associations studied show the same kind of departure from randomness in all populations. The explanation involving recent origin likewise seems unsatisfactory because the broad geographic range of most of the inversion associations indicates a very old, not a very recent origin.

In determining whether the associations of inversions within populations were random, expected numbers of each chromosome type were calculated; these numbers were based on the segmental frequencies within each population, the assumption of random association between segments and the assumption of no selective elimination of any particular chromosome type. The comparison between observed and expected chromosome types is summarized in Table 5. In the body of the table are listed the data from the seven largest population samples; included in the totals in the last two columns, but not given in the body of the table, are the similarly handled data from Petoskey, Mich., Iron River, Wis., and Iowa City, Iowa (24 chromosomes in all).

Table 5 shows that for the chromosome associations: + +·+ ++, A B·C BA and A B·C B+ there is a striking excess of observed over expected in every population sampled, and for all populations taken together. The above three associations were expected to occur in 151 chromosomes and were actually found in 322. On the other hand the remaining 21 classes of X chromosome associations listed in the table were expected to occur in 191 chromosomes and were actually found in only 20. All of the 21 associations which are either absent, or present in less than the expected frequencies, may be derived from the three associations present in excess by crossing over. Since it is clear that crossing over between the four segments actually occurs, then the low observed frequency of the 21 deficient types of chromosomes indicates their nonadaptive nature, and their continued removal from the population as they are produced by crossing over. It may be noted that while some of the deficient types may be derived by single crossovers from the three excessively frequent chromosome types, others would require a number of successive crossovers. The situation is presented in Chart 2 below. In this chart only combinations derived from the Standard + +·+ ++ and the common western A B·C BA are considered. A similar series may be derived from Standard and the eastern chromosome type A B·C B+, and differs from the series below only in the substitution of XR B+ for XR BA in each case.

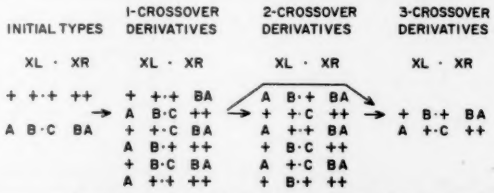

CHART 2.—Scheme to show the origin of recombinant X chromosomes from the Standard + +·+ ++, and the common western A B·C BA types. See text.

TABLE 5

Summary of X chromosome recombinations

Chromosome type XL · XR	St. Louis, Missouri		Mt. Vernon, Iowa		Plainwell, Mich.		Iron Mt., Mich.		Little Falls, Minn.		C.S.H., N.Y.		Bridgewater, Vermont		Totals	
	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
+ + · + ++	2.03	12	56.91	117	8.13	11	10.70	17	33.92	53	5.60	12	23.22	26	149.19	261
A B · C BA	1.28	11	0.35	31	0.002	1	0.01	3	0.03	9	0.02	0	...	..	1.70	56
A B · C B+	...	..	...	..	...	..	...	..	...	..	0.08	4	0.0001	1	0.07	5
A B · + BA	1.28	0	1.18	0	0.01	0	0.07	0	0.21	0	0.05	0	...	..	2.82	0
A B · + B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
A + · C BA	1.49	0	1.33	0	0.03	0	0.07	0	0.20	0	0.05	0	...	..	3.18	0
A + · C B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
+ B · C BA	1.49	0	1.33	0	0.03	0	0.05	0	0.18	0	0.05	0	...	..	3.14	0
+ B · C B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
A B · C ++	1.49	0	1.18	1	0.01	0	0.07	0	0.16	0	0.26	0	0.001	0	3.19	1
+ + · C BA	1.74	1	5.01	3	0.37	1	0.30	0	1.11	0	0.14	0	...	..	8.77	5
+ + · C B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
A + · + BA	1.49	0	4.52	0	0.10	0	0.42	0	1.23	0	0.14	0	...	..	8.01	0
A + · + B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
+ B · + BA	1.49	0	4.47	0	0.10	0	0.30	0	1.11	0	0.14	0	...	..	7.72	0
+ B · + B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
A + · C ++	1.74	0	4.51	0	0.17	0	0.42	0	0.99	0	0.71	1	0.04	0	8.68	1
A B · + ++	1.49	1	3.98	0	0.05	0	0.42	0	0.99	0	0.71	1	0.04	0	8.69	5
+ B · C ++	1.74	0	4.47	0	0.17	0	0.30	0	0.88	0	0.71	0	0.04	0	8.40	0
+ + · + BA	1.74	0	16.88	1	1.36	0	1.79	0	6.91	2	0.40	1	...	..	29.70	4
+ + · + B+	...	..	...	..	...	..	...	..	...	..	1.60	0	0.89	0	2.49	0
+ + · C ++	2.03	1	16.88	0	2.22	1	1.79	0	5.45	0	2.00	0	0.89	0	31.89	2
+ B · + ++	1.74	0	15.05	0	0.63	0	1.79	0	5.45	0	2.00	0	0.89	0	30.27	0
A + · + ++	1.74	0	15.05	0	0.63	0	2.52	1	6.17	1	2.00	0	0.89	0	31.72	2
Totals:	26.00	26	153.10	153	14.00	14	20.99	21	65.00	65	19.00	19	27.00	27	342.08	342

In this table a comparison is made between the observed and expected population frequencies of X chromosomes carrying the XL inversions A and B, and the XR inversions C and BA (thus A B · C BA), and chromosomes carrying none of these inversions (+ + · + ++) and all possible combinations between them. Chromosomes carrying the rare XL D and XR D and E inversions were not included. In order to shorten the table 24 X chromosomes from Petoskey, Iron River and Iowa City are not listed in the body of the table although they are included in the totals in the right hand column. The expected frequencies are worked out separately for each population on the basis of its own observed inversion frequencies and on the assumption that the four segments: *A B C BA* are separable by crossing over and should be associated randomly.

If the extremely unlikely double or triple crossing over is ruled out, then the 2-crossover and 3-crossover classes can only be derived via the 1-crossover classes. Thus adaptive inferiority of the 1-crossover classes would in itself tend to prevent production of the other derived classes. The observed and expected frequencies of the Initial types and the three derived classes from eight localities are listed in Table 6. The eight localities were chosen because of large sample size, and because of the fact that the samples studied carried at least two alternates in each of the four segments. The data in this table indicate that while all the derived classes are below expectation, this deficiency is particularly striking in the case of the 2- and 3-crossover classes. If the 17 derived types actually observed were randomly distributed among the three derived classes, then 47 percent or eight should have been 1-crossover types, and the remaining nine should have been 2- and 3-crossover types. The observed distribution of 14 1-crossover types and only three in the other two classes differs significantly from expectation. Using YATES' correction for continuity, $x^2 = 7.14$ and $P = < 0.01$.

DISCUSSION

The data and conclusions presented above on inversion associations within chromosomes may be briefly summarized. In both the second and X chromosomes the inversions show non random associations. This non randomness is especially striking in the X, and it is concluded that it is due to the relative nonadaptedness of certain inversion associations, which are eliminated as they are produced by crossing over. One may arrive at a rough estimate of the rate at which elimination of nonadaptive chromosomes must occur by a consideration of the rate of their production by crossing over within populations. For example, in the St. Louis, Mo., population approximately 50 percent of the females should be hetero-

TABLE 6

Frequencies of X chromosomes showing "initial types, 1-crossover, 2-crossover and 3-crossover derivatives" starting with Standard/A B · C BA or Standard/A B · C B+ as the initial types. Data from all sites showing two alternates in each of the four segments

Site	N	Initial types		1-crossover derivatives		2-crossover derivatives		3-crossover derivatives	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
St. Louis, Mo.	26	3.31	23	9.69	2	9.77	1	3.23	0
Plainwell, Mich.	14	8.13	12	2.44	1	3.15	1	0.27	0
Iowa City, Iowa	7	3.78	7	1.50	0	1.50	0	0.21	0
Mt. Vernon, Iowa	153	57.26	148	43.43	5	43.43	0	8.97	0
Little Falls, Minn.	65	33.95	62	15.52	3	13.43	0	2.10	0
Iron Mt., Mich.	21	10.71	20	5.14	1	4.42	0	0.72	0
C.S.H., N.Y.	19	5.69	16	7.94	2	3.94	0	1.43	1
Bridgewater, Vt.	27	23.22	27	1.85	0	1.85	0	0.07	0
Total:	332	146.05	315	87.51	14	81.49	2	17.00	1
Observed / Expected		2.157		0.160		0.025		0.059	

zygous + +·+ + +/A B·C BA and crossing over in such females leads to the production of the deficient types of X chromosomes. From the laboratory crossover data given above, the total proportion of single-crossover chromosomes produced by such a female would be the sum of the crossover values for each of the three sections of the X lying between inversions, thus 0.25 percent. Taking into account the noncrossover chromosomes produced by males, a population such as the one in St. Louis would be expected to produce nonadaptive X chromosomes at the rate of about 1/1,200 per generation. These would all be of the 1-crossover derived type, and might either be eliminated as such, or by further crossing over produce 2- or 3-crossover types which would be eliminated in their turn. In the northern part of the species range, where the frequency of the A B·C BA chromosome is lower, the rate of production of nonadaptive X chromosomes would be only about 1/2,400. Thus the price of maintaining Standard or A B·C BA chromosomes intact in the population is relatively low.

The cause of the relative nonadaptedness of the observedly deficient inversion associations is not clear at present, however one possible factor suggests itself. The A B·C BA and A B·C B+ X chromosomes carry the "sex-ratio" genotype. As has been pointed out by other workers, chromosomes carrying such a genotype should benefit from the effects of meiotic drive, since males carrying the sex-ratio chromosome tend to transmit it to all of their progeny (their daughters) while males with Standard X chromosomes transmit them to only the male half of their progeny. The fact that such sex-ratio chromosomes do not completely replace the Standard chromosomes (with possible extinction for the population) is due to the opposite effects of other selective forces (at present not well understood). Crossover X chromosomes carrying only part of the A B·C BA complex might be expected to lack the sex-ratio genotype, and thus lose the advantages of meiotic drive characteristic of the intact chromosome. Preliminary studies of crossover X chromosomes carrying part of the sex-ratio inversion complex indicate that some (but not all) of them do lack the sex-ratio genotype, and thus should suffer a reduction in their over-all adaptive value.

Non random association of inversions has been demonstrated, or is suspected for other species of Diptera, especially Drosophila (see LEVITAN 1958 for review). The species which has been most intensively studied in this respect is *D. robusta*, in which LEVITAN has presented data conclusively showing non random associations in the X and in chromosome 2. In this species the associations are of a somewhat simpler sort than those found in the X of *D. paramelanica*, and are in most respects comparable to those in chromosome 2 of the latter species, that is they involve adaptive associations between two segments of a given chromosome.

In *D. melanica* WARD (1952) has found that some of the inversions on chromosome 2 and on the X chromosome regularly occur together. LEVITAN cites this species as offering examples of non random associations, but it is the opinion of the author that he fails to prove his point in this particular instance. In addition to overlooking some rather crucial parts of WARD's data, he has listed examples

that are either represented by too few population data, or are tied up in inversion complexes that may not be separable by crossing over.

The very complex inversion associations demonstrated in the X chromosome of *D. paramelanica* bear some resemblance to the situation found in species of Drosophila in which adaptive associations of linked genes are maintained even without the help of inversion heterozygosity. In these cases the cost of maintenance of such associations is the elimination of individuals carrying nonadaptive chromosomes produced by recombination. Associations of this sort have been demonstrated in *D. melanogaster* (MISRO 1949; WALLACE *et al.* 1953), in *D. pseudoobscura* (DOBZHANSKY 1946; SPASSKY *et al.* 1958), in *D. prosaltans* (DOBZHANSKY *et al.* 1959) and in *D. persimilis* (SPIESS 1959).

In such species it might be expected that systems capable of producing lethal or semilethal recombinant chromosomes would not occur on the X chromosome. As stated by SPASSKY *et al.* (1958), "—one third of the X chromosomes in a population are carried in males which would die if they had a sex-linked lethal. Therefore, X chromosomes capable of giving sex-linked lethals by recombination with other X's in the same population would be discriminated against by natural selection. This is not necessarily the case with autosomes, since the lethals produced by recombination would be carried mostly in heterozygotes, and might be removed by another recombination. Only synthetic lethals that would kill homozygous females, but not males, are expected in the X chromosomes."

It is of course clear that any argument based on lethals will apply also to semilethals and gene combinations causing moderate reductions in viability or fertility. In the X chromosome of *D. paramelanica* recombinants are clearly eliminated. It is not clear in this case whether the elimination occurs predominantly in the male or the female, and the available data on wild populations are not sufficiently extensive to clear up this point.

One may think of the *D. paramelanica* X's as showing two types of adaptive genetic organization. On the one hand, X's which when in structural *homozygotes* are able to produce recombinant lethals or semilethals should be discriminated against, and might be expected to be absent from the population. However, X chromosomes which produce lethals or semilethals following rare crossing over in the structural *heterozygotes* would suffer so little from selective elimination of males, that they would be expected to survive in a population. It is not surprising, therefore, that adaptive inversion associations occur on the X chromosomes of a number of other species of Drosophila. They are found in the *D. obscura* group (where they are associated with the sex-ratio trait), in *D. americana* (BLIGHT 1955) and in *D. robusta* (CARSON and STALKER 1949; LEVITAN 1958).

SUMMARY

1. The paper describes a survey of chromosomal polymorphism in *Drosophila paramelanica*, a member of the *D. melanica* species group found in northeastern North America. Samples of this species were taken from 18 widely separated localities, and the chromosomes from the various populations were classified by

means of crosses with a laboratory Standard strain originating in St. Louis, Mo. Approximately 370 X chromosomes and 600 of each kind of autosome were analyzed from wild populations.

2. Only the X chromosome and chromosome 2 were found to be structurally polymorphic. In the rod-shaped chromosome 2 seven inversions differing from Standard were discovered, and a total of 16 gene sequences, including Standard, have been found in nature. In the V-shaped X chromosome four inversions were found in the left arm and five were found in the right. For the whole chromosome 13 gene sequences including Standard have been found in nature.

3. In chromosome 2 the known inversions are located primarily at the base or tip. The gene sequences in either region show clinal changes in frequency, with certain patterns predominantly northern, others southern. In an Iowa population lying between the northern and southern parts of the species range, where both northern and southern arrangements occur together, the distal and proximal arrangements are not associated at random, but tend to be associated: North-North or South-South.

4. In the X chromosome a series of short inversions tend to be associated; they are inversions A and B in X-left and C and B in X-right, thus: A B·C B. It has been shown that these inversions are all separable by rare crossing over. The commonest kinds of X chromosomes in all populations are either Standard (+ +·+ +) or those with inversions A B·C B. The X's which might be produced by crossing over and subsequent separation of these four inversions are either absent, or present in frequencies significantly below expectation. This relationship holds in all populations studied.

5. Of the X chromosomes showing only some of the four inversions, those which might be produced from A B·C B/+ +·+ + females by a single crossover (such as A B·C + or A +·+ +) are much more frequent than those which would require a succession of single crossovers (such as A +·C B or A B·+ B).

6. It is concluded that natural selection is eliminating the deficient types of X chromosomes as rapidly as they are produced by crossing over (predominantly in ++·++/A B·C B females). Since even the types of chromosomes which may be produced by a single crossover are deficient in the population, it is suggested that the greater deficiency of types that must be produced by two or more successive crossovers can be ascribed to the initial nonadaptedness of the 1-crossover types which makes subsequent production of the 2- and 3-crossover types highly improbable.

7. Preliminary laboratory tests of crossing over in heterozygous A B·C B/ ++·++ females indicate that in a population in which the A B·C B chromosomes have a frequency of 40 percent, as in Missouri, approximately 1/1,200 of the X-bearing gametes formed should show some type of recombination for the four inversions. More northern populations would be expected to produce about 1/2,400 such gametes. Thus the cost to the species of maintaining the adaptive association is a low one.

8. All A B·C B X chromosomes tested carry the sex-ratio trait, of the sort found

in the *D. obscura* group, and thus males carrying such chromosomes tend to produce only daughters. In *D. paramelanica*, as in the *D. obscura* group, sex-ratio X chromosomes have a higher frequency in the southern part of the range (40 percent) than in the north (nine percent).

9. The V-shaped chromosome of *D. paramelanica* apparently includes STURTEVANT and NOVITSKI's Element A (corresponding to the rod-shaped X of *D. melanogaster*) and some other element which is autosomal in *D. melanogaster*. Since in *D. paramelanica* X-right carries the mutant singed apparently this arm should correspond to Element A; the identity of the other element is not clear.

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THE PALE GREEN MUTABLE SYSTEM IN MAIZE[1]

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IN 1938 (and 1941) RHOADES reported the case of a mutational event controlled by a specific intranuclear factor (the Dotted gene)[3] which causes the recessive a_1 allele to mutate to the dominant form A_1. Since then additional evidence for specific genic controllers of mutation has been reported by MCCLINTOCK (1950, 1951) and BRINK and NILAN (1952). Their work has shown that mutable genes are governed by accessory factors. These same factors are readily transferable to various locations in the genome and are able to regulate the time and rate of gene mutation. It was further shown that these factors of mutable systems can, when transposed, govern the action and mutability of other loci (MCCLINTOCK 1953).

The current report is an analysis of the pale green mutable gene system and its various components. This system is similar in many ways to previously described systems and, in addition, there is evidence for an even closer relationship of component parts. An abstract of the material in this report has been presented by PETERSON (1953).

MATERIALS AND METHODS

Pale green mutable (pg^m) was found by E. G. ANDERSON among material exposed to the Bikini A-bomb. Mutant plants from segregating ears were crossed to standard inbreds. These F_1 plants were both selfed and outcrossed to standard inbreds again. Such recurrent outcrossing was practiced successively for a number of generations.

Two distinct mutant types, pg^m and pg^s, appear among the F_2 progeny of pale green mutable outcrosses (Figures 1 and 2).

These pg^m seedlings are characterized by numerous dark green stripes on a pale green background. As will be shown later, these stripes represent mutations of pale green to green ($pg \rightarrow Pg$).

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[2] In part adapted from a portion of a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Illinois. Additional data obtained at the University of California, Riverside, and Iowa State College.

[3] A number of abbreviations used in the text are described:

Dt—Dotted gene that causes the a_1 allele (colorless aleurone) to mutate to A_1 (colored aleurone).

Ac—Activator described by MCCLINTOCK that causes *Ds* (the Dissociator locus) to change.

Spm—Suppressor-mutator described by MCCLINTOCK that suppresses the action of a locus such as a_1 and also causes it to mutate.

v—Refers to the variegated flower locus in Nicotiana.

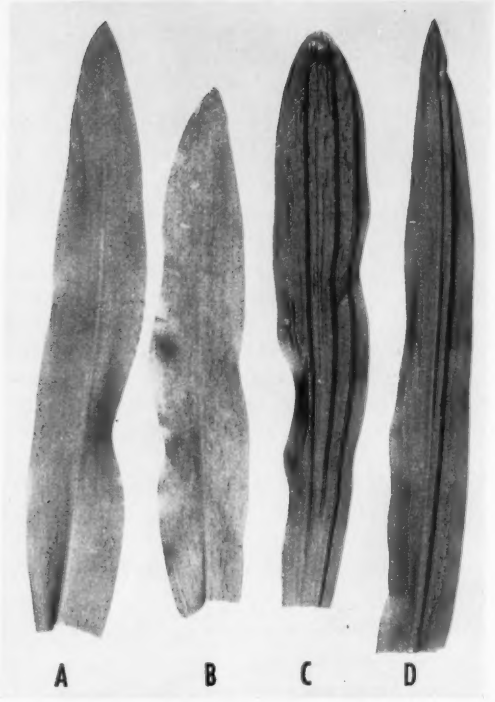

FIGURE 1.—Seedling leaves of mutant plants. A.—pg^s; B, C and D—pg^m; B.—very late occurring mutant stripes; C.—early and late occurring mutant stripes; D.—early occurring mutant stripes.

There are various gradations of variegation (Figures 1B, 1C, and 1D) which depend upon the time of the mutation event (early or late in plant development). In most cases these differing patterns are a function of the inherited properties of the allele. In general, intercrosses of early and late pattern types show the former to be dominant. Mutable seedlings grow vigorously in a sand bench and in the field, some reach maturity and produce pollen and ears.

The pg^s seedlings represent the stable-type pale green allele and possess only an occasional stripe or sector of mutability (Figures 1 and 2). pg^s plants grow as vigorously as pg^m.

RESULTS AND DISCUSSION

The three classes of pale green segregating F_2 progenies

The two phenotypes, pg^s and pg^m, appear together with the normal green plants in three characteristic ratios in segregating F_2 progenies of outcrosses of

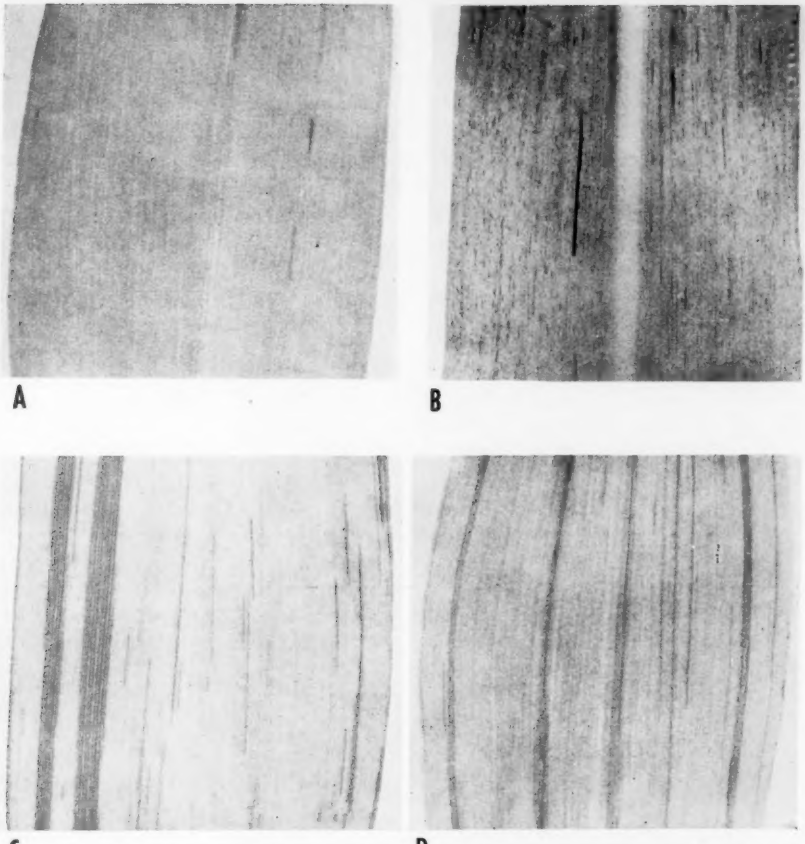

FIGURE 2.—Close-up photographs of mutant seedlings. A.—pg^s with sector of mutability. B, C, and D—pg^m; B.—very late occurring mutant stripes; C.—early and late occurring; D.—early occurring mutant stripes.

heterozygous mutable ($+/pg^m$) plants. These F_2 progenies represent three different classes—the m. m and s, and s (Table 1).

These class designations are based on the proportion of pale green seedlings that are stable. The three classes are as follows:

s = 3 green : 1 pg^s
m and s = 12 green : 3 pg^m : 1 pg^s
m = 3 green : 1 pg^m + low frequency of pg^s

Plants from these three types of F_2 progenies were used in further crosses.

The data to be presented in this paper support the hypothesis that the mutable

TABLE 1

F_2 progenies (1957 527 and 1957 528) arising from the outcross of an autonomous pg^m *allele to a standard line showing the segregation of* pg *that distinguishes m, m and s, and s classes*

Standard × 1956 464–3 → 1957 527; Standard × 1956 464–32 → 1957 528; +/+ × +/pg^m → selfed *pg*-segregating progeny

1957	*Pg*	Total *pg*	pg^s	pg^m	Classes s	m and s	m
527–3 ⊗	69	18	0	18	..	..	1
–4 ⊗	138	37	0	37	..	..	1
–33⊗	134	37	0	37	..	..	1
–34⊗	113	33	2	31	..	..	1
–36⊗	117	28	0	28	..	..	1
–37⊗	122	43	9	34	..	1	..
–60⊗	154	50	3	47	..	..	1
–62⊗	141	46	2	44	..	..	1
–63⊗	59	30	0	30	..	..	1
–64⊗	89	20	1	19	..	..	1
–65⊗	188	42	2	40	..	..	1
–67⊗	91	35	0	35	..	..	1
–71⊗	81	26	0	26	..	..	1
Total	1496	445			0	1	12
Total = 1941		in m classes	10	432			
Percent *pg* = 22.92							

1957	*Pg*	Total *pg*	pg^s	pg^m	Classes s	m and s	m
528–6 ⊗	106	14	0	14	..	..	1
–7 ⊗	64	20	0	20	..	..	1
–8 ⊗	183	34	2	32	..	..	1
–9 ⊗	94	15	0	15	..	..	1
–10⊗	81	19	0	19	..	..	1
–12⊗	59	16	4	12	..	1	..
–13⊗	245	44	10	34	..	1	..
–17⊗	81	14	0	14	..	..	1
–18⊗	82	23	0	23	..	..	1
–26⊗	130	11	0	11	..	..	1
–27⊗	91	9	2	7	..	1	..
–28⊗	78	20	6	14	..	1	..
–29⊗	76	21	0	21	..	..	1
–30⊗	66	23	4	19	..	1	..
–33⊗	115	26	1	25	..	..	1
–36⊗	67	21	21	0	1	..	..
–61⊗	92	15	0	15	..	..	1
–62⊗	210	66	2	64	..	..	1
–65⊗	193	41	11	30	..	1	..
–66⊗	352	39	4	36	..	..	1
–68⊗	128	38	0	38	..	..	1
–69⊗	139	35	0	35	..	..	1
–71⊗	137	45	0	45	..	..	1
–72⊗	137	26	0	26	..	..	1
Total	3006	635			1	6	17
Total = 3641		in m classes	9	453			
Percent *pg* = 17.4							

Pg = green
pg^s = stable pale green
pg^m = mutable pale green
⊗ = selfed

pg system consists of two accessory components. One component, *I*, inhibits the expression of the normal green allele, *Pg*, and causes the pale phenotype to be expressed. The removal of *I* (the mutation event) is controlled by a second factor, Enhancer (*En*), that can be variously located; at the *pg* locus where control is autonomous (m class) and on chromosomes independent of *pg* (m and s class). In the absence of *En*, the *pg* seedlings are stable.

The m and s class: F_2 populations segregating 12 green : 3 pg^m : 1 pg^s, arose from two types of outcrosses: from green plants heterozygous for pg^m (Table 1, 6) and from some homozygous mutable plants (Table 2). (G families listed in Table 2 contain only green seedlings and arise from the mutation of *pg* to *Pg*.) Such a modified two-pair ratio indicates that a factor controlling the mutability of *pg* is assorting independently—when present, *pg* is mutable, when absent, the allele is stable (pg^s). This is similar to the two unit interaction of the Dotted gene (*Dt*) on a_1 (RHOADES 1938, 1941) and of *Ac* on *Ds*—controlled loci (MCCLINTOCK 1950, 1951). In view of its ability to make *pg* mutable, this factor is called Enhancer (*En*). Thus, the F_1 genotype that gave rise to the m and s type class is designated *En*/+, +/*pg*.

When these F_1 plants are further outcrossed to normal lines and segregating families subsequently obtained, two classes of *pg* segregating progenies appear—s, and m and s, which occur in a 1:1 ratio ('57 805–10 and 806–18 in Table 3). This ratio shows that one half of the *pg* containing gametes of the F_1 plants possessed *En* and one half were without *En*.

Further confirmation of the presence of such a factor assorting independently of *pg* is obtained by F_3 tests. Results of these crosses (Table 4) confirm the fact that there are two units in this mutable system, the *pg* allele and *En* which when present causes *pg* to be mutable. The progenies of these F_3 tests segregate as fol-

TABLE 2

Summary of F_2 progenies resulting from the outcross to various inbred lines of two mutable plants homozygous for independent En

Pollen parent	F_1 family	Classes of F_2 progenies† G	s	m and s	m
1950 43–54 pg^m/pg^m	51 199	0	0	2	0
	51 226	1	0	2	0
	51 229	3	0	18	2
1950 43–57 pg^m/pg^m	51 200	1	0	1	0
	51 202	2	0	6	1
	51 696	1	0	5	1*
	51 418	10	0	17	0
	Total	18	0	51	4

* This m type was outcrossed and tested further as 1957 530–4 and 530–5 (Table 6).
† The three class designations are based on the proportion of pale green seedlings that are stable from selfed progeny.
s = 3 green : 1 pg^s
m and s = 12 green : 3 pg^m : 1 pg^s
m = 3 green : 1 pg^m : with a low frequency of pg^s
G families contain only green seedlings and arise from the mutation of *pg* to *Pg*.

TABLE 3

Summary of F_2 progenies resulting from outcrosses of plants that give m and s, and s progenies to standard inbreds

Pollen parent Plant	Segregation from self $Pg : pg$	$(pg^s : pg^m)$	Class	F_1 family	G	Classes of F_2 progenies s	m and s	m
Outcross of m and s								
1957 805–10⊗	146 : 31	8 : 23	m and s	1958 1260	29	14	9	0
1957 806–18⊗	107 : 30	9 : 21	m and s	1958 1267	14	7	9	0
Outcross of s								
1957g 9–16⊗	81 : 17	17 : 0	s	1957 804	24	20	0	0

TABLE 4

F_3 progenies obtained from the self-pollination of F_2 green plants occurring in m and s F_2 progenies

F_2 families	Types of pale green segregating among the progeny Only pg^s (++)	pg^m and pg^s (*En* +)	Only pg^m (*En En*)	Total
1951 97–100, 273–274	10	15	7	32
1952 292A	5	8	2	15
1956 431–432	7	12	7	26
	22	35	16	

lows: ¼ only pg^m (*En En*): ½ ¾ pg^m to ¼ pg^s (*En* +): and ¼ only pg^s (++).

The stable type progenies that give only stable seedlings can become mutable when crossed to *En* bearing stocks (Table 7). *En* has no obvious effect in the absence of *pg*. Furthermore, in appropriate tests *En* was found to be ineffective in activating *Ac* or *Dt*–controlled loci.

Proof for the mutation event—pale green to green—is obtained from the outcross of homozygous mutable plants to inbred lines (Table 2). Among the 73 F_2 progenies, there were 18 not segregating *pg*. These possess the *Pg* allele, a result of the *pg* to *Pg* mutation, and remain stable. The high proportion of *Pg* alleles (18/73) from pg^m/pg^m plants arises from early mutation which in some cases would include the sporogenous tissues. Appropriate genetic markers have been used in these crosses to rule out the possibility of contamination.

The s class: The s class comprises the F_2 progenies that segregate 3 green to 1 pg^s (Table 5). That *pg* is a simply inherited recessive is indicated by the 25 percent of *pg* plants. This s class is observed as an exceptional F_2 progeny type derived from the outcross of $+/pg^m$ plants (Tables 1 and 6). In further outcrosses of such types (57g 9–16 in Table 3) only s type progenies result.

Seedlings of the s class lack *En*. Some pg^m seedlings (1/512) do arise among s type F_2 progenies. This suggests a reversion to the mutable form (Figures 2A and 3). The significance of this event will be discussed in a later section.

The m class—the autonomous Enhancer: In contrast to the above mentioned

TABLE 5

Segregation of pg *seedlings among F_2 progenies of the s class*

	Pg	*pg*^{s}	*pg*^{m}	Total	Percent *pg*
1951–1952 families	8590	2930	7	11,520	25.5
1957 families	1878	657	0	2,535	25.9
Total		3587	7		

pg^m = 1/512 = 0.19 percent.

TABLE 6

The classes of F_2 progenies arising from the first and second generation outcrosses of m-type plants to standard inbreds

Source of *pg*^{m} +/*pg*^{m}	Segregation from self of +/*pg*^{m} *Pg*:*pg*	(*pg*^{s}:*pg*^{m})	F_2 progeny family	G	Classes of F_2 progenies s	m and s	m*	Total	Freq. of non-m type
1st generation outcrosses 1957									
1956 464–3	175:39	3:36	527	26	0	1	12[a]	13	1
1956 464–32	103:26	0:26	528	18	1	6	17[b]	24	7
1956 464–59	74:15	0:14	529	15	0	2	14[c]	16	2
1956 474–10	146:22	0:22	530	26	2	3	14[d]	19	5
1956 476–16	72:17	0:17	531	15	0	0	8	8	0
1956 464–35	50:20	0:20	535	28	0	3	11	14	3
1956 465–1	126:20	0:20	536	23	1	2	6	9	3
1956 g9–19	70:17	0:17	803	25	0	5	11	16	5
1956 g9–12	89:24	0:24	805	54	6	24	29[e]	59	30
1956 g9–7	84:21	0:21	806	59	6	16	25	47	22
					16	62	147	225	
				Percent	7.11	27.56	65.33		
				Percent expected with 3 *En*	12.56	37.50	50		
2nd generation outcrosses 1958									
1957 527–4	138:37	0:37	1269	23	6	8	11	25	14
1957 527–33	134:37	0:37	1270	30	1	8	11	20	9
1957 527–36	117:28	0:28	1272	29	1	7	9	17	8
1957 528–6	106:14	0:14	1273	37	2	6	12	20	8
1957 528–10	81:19	0:19	1275	33	2	2	9	13	4
1957 528–17	81:14	0:14	1277	16	2	5	7	14	7
1957 528–29	76:21	0:21	1279	20	1	4	18	23	5
1957 528–33	115:26	1:25	1281	25	5	5	12	22	10
1957 529–1	169:30	0:30	1282	17	2	3	8	13	5
1957 529–7	162:21	0:21	1283	26	1	1	9	11	2
1957 529–30	105:17	0:17	1284	34	1	1	13	15	2
					24	50	119	193	
				Percent	12.4	25.9	61.7		
				Percent expected with 3 *En*	12.50	37.50	50		

* Some of the m types of this family were retested and gave rise to a-1269–72, b-1273–81, c-1282–84, d-1285–86 and e-1258–63.

FIGURE 3.—pg^s seedlings which contain some stripes and sectors of mutability. A faint individual stripe that is commonly found in pg^s seedling leaves is circled.

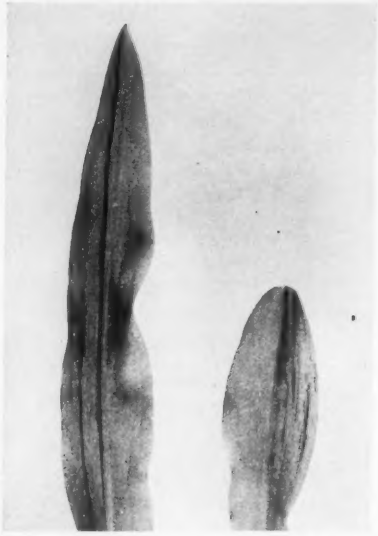

FIGURE 4.—Stable sectors in the leaves of pg^m plants; these may represent the somatic loss of *En*.

s, and m and s type F_2 progenies, a third—the m type—was most frequent (Tables 1 and 6). In these m type F_2 progenies all the pale green seedlings are mutable except for a small percentage (0–10 percent) of stables. Two such families (527 and 528 of Table 6) are shown in Table 1. The m class predominates among the F_2 progenies of second generation outcrosses of m type plants and continues to give rise to some s and m and s type F_2 progenies. In further tests these exceptional types continue to behave as s, and m and s types (Table 3).

The simplest explanation of why in the m class all the *pg* seedlings are mutable is that *En* is located next to the *pg* locus or is a component of it so that mutability is apparently autonomously controlled (designated $\widehat{pgEn}$). The *En* element at the *pg* locus resembles the independent *En* since both control mutability although they are located in different positions.

From the data in Table 6 it can be concluded that the F_2 progenies from outcrossed mutable plants, whose mutability is controlled by autonomous *En*, are predominately m type although s, and m and s type progenies also arise. The frequency of these latter types is interpreted as indicative of a high rate of change from $\widehat{pgEn}$ (m class) to *pg* with independent *En* (m and s class) and to *pg* with no *En* (s class). This is significant since it relates the independent *En* to the autonomous.

There is an alternative that should be considered in attempting to explain the

m class and the origin of the resulting outcross derivatives. Could the low frequency of stables among m type F_2 progeny be explained by the segregation of more than one independently assorting Enhancer as was found by McCLINTOCK (1956) for *Spm*? In the self of a heterozygote for *pg* and *En* (*pg*/+, *En*/+), a 3 mutable to 1 stable ratio is obtained among the pale green seedlings due to the segregation of *En*. If *En* is present in duplicate (*En* at two independent locations) $En_1/+$, $En_2/+$ the frequency of non-*En* pale green or stable seedlings is 1/16. In the triplicate condition, $En_1/+$, $En_2/+$, $En_3/+$, only 1/64 of the resulting pale green seedlings would lack an *En* and be stable. Therefore, the frequency of pg^s under the influence of many Enhancers would simulate the m type progeny and could not under the present crossing procedure be readily recognized in an F_2 progeny.

In order to distinguish between this possibility and the hypothesis of highly transposable autonomous *En*, a recurrent outcross program was begun to test the independent *En* composition of heterozygous *pg* plants (+/*pg*). After one generation of outcrossing to inbreds, heterozygous plants (+/pg^m) that yield m type F_2 progeny were also outcrossed to standard lines and F_2 progenies were subsequently obtained. These F_2 progenies were then classified as s, m and s, and m (Table 6-second generation outcrosses). If *En* were present in duplicate, the outcross of such a genotype to a standard inbred ($En_1/+$, $En_2/+ \times +/+$, $+/+$) with regard only to *En*, would yield F_2 ears in the following ratio: ¼ s (no *En*): ½ m and s (1 *En*): ¼ m (2 *En*). If the m class were due to the presence of *En* in the triplicate condition ($En_1/+$, $En_2/+$, $En_3/+$), the outcross of such a genotype would yield 1/8 +/+, +/+, +/+; 3/8 *En*/+, +/+, +/+; 3/8 *En*/+, *En*/+, +/+ and 1/8 *En*/+, *En*/+, *En*/+. Since the latter two genotypes would yield 1/16 and 1/64 pg^s seedlings respectively, their progeny are grouped into the m class. This results in a combined ratio of 1/8 s: 3/8 m and s: 4/8 m.

The above ratios were not obtained in the progeny testing of m type parents (Table 6). In the first generation outcrosses some parents such as 1956 464–3, 464–59 and 476–16, gave only a low frequency of non-m types (s, and m and s). Half the gametes of 1956g 9–12 and 9–7 (Table 6) were of the non-m type. In the second generation outcrosses, 1957 plants 528–29, 529–7 and 529–30 gave low frequencies of non-m type F_2 progenies while the other plants gave a higher rate. The general heterogeneity of the data with respect to the frequencies of s, and m and s derived progenies of the different plants may be complicated by the presence of nonautonomous *En* on the same chromosome but not adjacent to *pg*. In any case the expected ratios of the three classes based on the presence of three *En* are given in Table 6 for each generation of outcrossing. These expectations do not agree with the results obtained. It is also obvious that they do not fit a 1 s:2 m and s:1 m ratio which would be expected if *En* were present in two independent positions.

The alternatives can be further tested by comparing the frequency of expected pg^s with the observed in F_2 progeny if three *En* are assorting independently. It was previously mentioned that with three *En* (*En*/+, *En*/+, *En*/+) 4/8 of the F_2

ears should be of the m class. In this case 3/4 of the m class would be *En*/+, *En*/+ and would yield 6.25 percent stables and ¼ would be *En*/+, *En*/+, *En*/+ yielding 1.56 percent stables. The combined yield of stables would therefore be 5.07 percent [i.e., (¾ × 6.25% = 4.08%) + (¼ × 1.56% = 0.39%) = 5.07%]. Two families were chosen to test this. In family 1957 527 the observed occurrence of pg^s is ten [expected 22.6 (5.07% × 442)—P=0.02] while in 1957 528 the observed is nine [expected 22.9 (5.07% × 462)—P = 0.01] (Table 1). Again the observed frequency does not agree with the expected if *En* is assorting independently in triplicate.

Another test can be made to distinguish between the multiple *En* and autonomous *En* alternatives. The non-*pg* segregating plants arising from the outcross of +/$\widehat{pgEn}$ are crossed by a known pale green stable that responds in the presence of *En*. If the m class is due to autonomous *En*, the subsequent F_2 ears should yield only s type progenies. If, however, many *En* are present in the background, m, m and s, and s type classes should arise. The preliminary results from such crosses indicate that *En* must be autonomous since most of the F_2 progenies segregate only stables. This does not exclude the possibility that an occasional independent *En* is present in autonomous stocks. Extensive crosses are underway to test more fully the independent *En* composition of autonomous stocks.

From these experiments it is clear that the m class is not due to the presence of independently assorting Enhancers at two or three loci. The hypothesis that *En* is autonomous is considered to be valid. The occurrence of stables in a low frequency results when *En* moves from the *pg* locus. This transposition of *En* accounts for the appearance of the s, and m and s classes. The s class would result from the exclusion of *En* in a *pg* gamete while the m and s class would result from the inclusion in a gamete of *En*, independent of *pg*. Stable sectors occur somatically which may indicate the loss of *En* (Figure 4).

The possible presence of independent *En* in the background of some autonomous stocks makes difficult the determination of the exact rate of change of pg^m to pg^s. The best estimate can be obtained by analyzing the rate of change in the m class to the s, and m and s types (Table 6). In these tests there are 153 s, and m and s class ears in a total of 419. This rate of change (36.7 percent) is indicative of very high rate of transposition of *En* from the *pg* locus. The rates and direction of change are illustrated in Figure 5. This does not, of course, consider the effect of early mutations upon sporogenous tissue.

The rate of change of $\widehat{\text{pgEn}}$ *to* pg *and* pg + En: As stated above the frequency of s versus m and s type progenies would be difficult to predict if any independent *En* were present in the autonomous stocks. If the change of $\widehat{pgEn}$ to *pg* without *En* is simultaneous with its incorporation into some part of the genome, then one would expect a 3 s: 1 m and s ratio of the two classes; (the chance of the inclusion of transposed *En* with the *pg*-containing cell in somatic mitosis rather than with the $\widehat{pgEn}$-containing cell plus the additional chance of its noninclusion with *pg* during assortment in meiosis would lead to such an expected ratio). There is a higher proportion of the m and s class and this deviation from the expected 3 s : 1

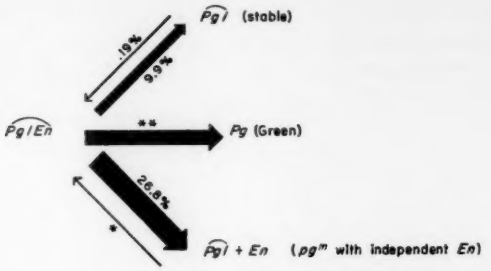

FIGURE 5.—The direction and relative rates of change of $\widehat{PgIEn}$ gametes.

* These exceptional progeny have not all been tested but the rate of change in this direction appears higher than the change from $\widehat{PgI}$ to $\widehat{PgIEn}$.

** An absolute rate cannot be determined due to early mutations affecting sporogenous tissue.

m and s ratio (Table 6) may indicate that the transposition event occurs late in microsporogenesis so that *En* is included in the same cell with *pg*.

Dual position of En *and the nature of* pg^s

In a preceding section the pale green mutable controlled by an independent Enhancer was discussed. From this type plant, heterozygous both for *pg* and *En*, (*Pg*/*pg*, *En*/+) a stock segregating only mutable seedlings was obtained (*Pg*/*pg*, *En*/*En*). Further selfing yielded a stock not segregating *pg* but homozygous for *En*. This stock, (*Pg*/*Pg*, *En*/*En*) was used as an *En* tester and was maintained by selfing for a number of years.

In order to test the effect of this stock on different *pg*^{s} alleles, stable pale green plants were crossed to this *En* tester stock. If *En* will cause *pg*^{s} to mutate, all F_2 ears obtained from such crosses (*Pg*/*Pg*, *En*/*En* × *pg*/*pg*, +/+) should segregate 12 green: 3 *pg*^{m}: 1 *pg*^{s}, the ratio obtained from m and s type segregation. Out of 78 pale green segregating progenies from this cross, 71 were of the m and s type (Table 7).

Thus it is evident that a factor independent of the *pg* locus, but controlling its mutability, is present in this stock. In similar crosses of *pg*^{s} to unrelated genetic tester and agronomic lines the presence of *En* was never detected.

In addition to the m and s class, two of the F_2 progenies were of the s class. This suggests the loss of the *En* factor in some of the gametes of the original *Pg*/*Pg*, *En*/*En* parent. In further tests *En* proved to be absent.

The remaining five progenies segregated only a small percentage of *pg*^{s} (3.8 to 8.1 percent) among the pale green seedlings (Table 8). These ratios are characteristic of duplicate factor ratios (expected 6.25 percent); therefore *En* must be heterozygous at two different and independent locations. The self of such a plant (*En*$_1$/+, *En*$_2$/+) would yield a wide array of F_2 types in terms of the *En* factors (Table 9). When these F_2 types are crossed by *pg*^{s}, the resulting progenies should yield the following ratio with reference to the segregation of *pg*^{s} among the pale

TABLE 7

F_2 progenies arising from the cross of Pg/Pg,En/En × Pg/pg (*no* En)

1956	G	F_2 families s	m and s	m
433	4	0	3	0
435	4	0	5	2
437	4	0	4	0
441	8	1*	8	0
442	10	0	7	0
443	7	1	10	2†
444	9	0	6	0
445	8	0	9	0
447	6	0	8	1
448	12	0	7	0
450	5	0	4	0
Total		2	71	5

* In further tests *En* found to be absent.
† In further tests two *En* found to be present.

TABLE 8

Exceptional progenies (see Table 7) from the cross Pg/Pg,En/En × Pg/pg^s, +/+

	Pg	*pg*	pg^s	pg^m	pg^s/total *pg* percent
435–5 ⊗	181	56	4	52	7.1
435–12⊗	137	37	3	34	8.1
443–10⊗	132	35	2	33	5.7*
443–13⊗	145	49	3	46	6.1
447–10⊗	131	26	1	25	3.8

* Tested by crossing by pg^s.

TABLE 9

Possible genotypes from the self of $En_1/+En_2/+$ *and expected segregation in crosses by* pg^s

Possible genotypes	Segregation in crosses by pg^s	
1/16 $En_1/En_1, En_2/En_2$ 2/16 $En_1/En_1, En_2/+$ 2/16 $En_1/+/+, En_2/En_2$ 1/16 $En_1/En_1, +/+$ 1/16 $+/+, En_2/En_2$	7/16 would yield only mutable seedlings	= Type A
4/16, $En_1/+, En_2/+$	4/16 would yield 3 pg^m : 1 pg^s	= Type B
2/16 $En_1/+, +/+$ 2/16 $+/+, En_2/+$	4/16 would yield 1 pg^m : 1 pg^s	= Type C
1/16 $+/+, +/+$	1/16 would yield only pg^s	= Type D

Type	A	B	C	D	Total
Observed	11	10	11	4	36
Expected 7:4:4:1 ratio,	15.75	9	9	2.25	36 P = .35

green seedlings: 7/16–no pg^s: 4/16–1/4 pg^s: 4/16–1/2 pg^s: 1/16–all pg^s (Table 9). One of the F_2 progenies (1956 443–10) was tested by crossing a large number of F_2 green plants by pg^s and the ratio of pg^m:pg^s among the resulting progenies was determined. As is evident in Table 9, the actual values agree with those expected when two *En* are assorting independently. This second location may be coincident with the absence of *En* in some gametes (Table 7).

The cross of pg^s by an *En* tester stock also provides information about the nature of the pg^s allele. pg^s is stable in the absence of *En*, but in its presence, mutates from pale green to green at a high rate. Could pg^s be similar to the quasi-stable forms of some mutable systems (McCLINTOCK 1951) that respond to a high dosage of the mutator by causing mutations to occur very late? This lateness results in the absence of observable mutations and gives the appearance of a stable phenotype. Under this mode of action a decrease in the dosage of the mutator results in visible mutability, which is what would be expected in outcrosses of stables in the case of pg^s. However, in such outcrosses, pg^s remains stable. Furthermore, the cross of pg^s to an *En* stock would only accentuate the stable condition among the progeny, if pg^s is due to high dosage of *En*. It does result, however, in mutability and supports the hypothesis that pg^s is due to the absence of *En*.

The pg^{mo} *allele*

Among 22 F_2 ears arising from the outcross of one mutable plant, 21 yielded the usual type of mutable seedlings but the other F_2 ear segregated a strikingly different, mottled-type seedling (Figures 6 and 7). Upon further testing this mutant was found to be allelic to *pg* and has been designated pg^{mo}. The seedlings of pg^{mo} contain very heavy, indistinctly outlined mutant stripes. These contrast with the clear-cut, straight line stripes that are characteristic of pg^m seedlings. The change from pg^{mo} to *Pg* (green areas) causes considerable deviation in the expected 3 *Pg*: 1 pg^{mo} F_2 segregation (Table 10).

In order to test the inheritance pattern of the pg^{mo} allele, pg^{mo} was outcrossed to standard lines and 19 F_2 progenies were obtained. All the pale green seedlings of these F_2 progenies were pg^{mo} which indicates that the control of mutability resides at the *pg* locus and is not determined by an independent factor. This allele was used in other tests.

The heterozygotes (pg^m/pg^{mo}) resulting from crosses of pg^{mo} to mutable plants express the pg^m pattern and show no evidence of the distinctive pg^{mo} type expression. In crosses of pg^{mo} to pg^s, however, all the seedlings are stable (Figure 6B). In the heterozygote, pg^{mo}/pg^s, the pg^s allele in some way completely inhibits the expression of the pg^{mo} allele.

A clue to this inhibition and to the hypothetical nature of the *pg* locus appears in crosses of pg^{mo} with the *En* tester stock. In the F_2 progeny of the cross (*Pg/Pg*, *En/En* × pg^{mo}/pg^{mo}, +/+) the expected ratio of green to pale green occurred, but among the pale green a ratio of 3 pg^s: 1 pg^{mo} resulted (Table 11). In this cross, only one *pg* allele, pg^{mo}, is being tested with *En*. The ratio of 3 stable : 1 pg^{mo} among the pale green seedlings clearly indicates that the *En* factor inhibits the

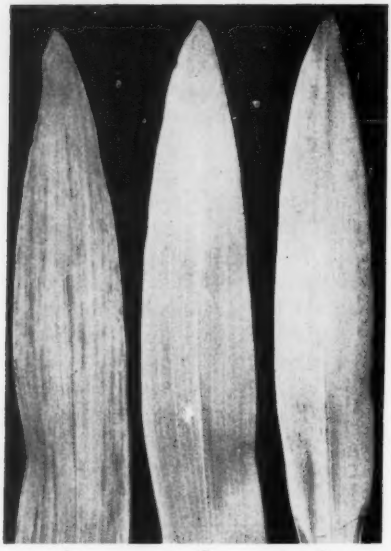

FIGURE 6.—*pg*-mutant seedings. A.—*pg*^{mo}. B.—*pg*^{mo} + *En* grown at 65°–70°F; C.—*pg*^{mo} + *En* grown at 95°F or above.

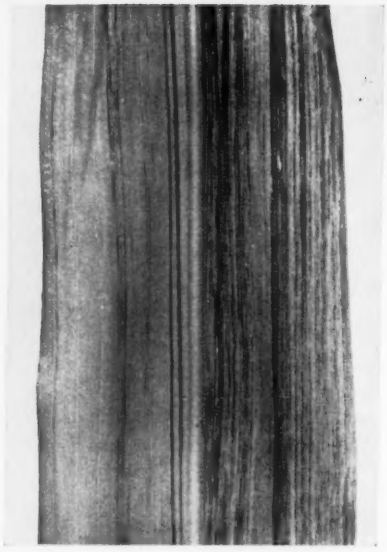

FIGURE 7.—Close-up view of a *pg*^{mo} seedling leaf.

TABLE 10

Some sample segregations in F_2 progenies arising from the outcross of pg^{mo} *to standard lines*

	Green	*pg*^{mo}
1956 559–1⊗	42	3
–2⊗	77	11
–3⊗	21	6
–6⊗	38	12

TABLE 11

F_2 progenies arising from the cross pg^{mo}/pg^{mo} *and* Pg/Pg.En/En

1957	*Pg*	*pg*	*pg*^{s}	*pg*^{mo}
567–6 ⊗	39	13	9	4
567–11⊗	48	10	8	2
569–5 ⊗	42	14	12	2
569–8 ⊗	64	18	13	5

expression of the pg^{mo} allele ($\frac{3}{4}En/-,pg^{mo}/pg^{mo}$) (Figure 6B), while in the absence of *En* it is fully expressed ($\frac{1}{4}$ +/+, pg^{mo}/pg^{mo}).

The inhibition of pg^{mo} expression by *En* is not absolute. In mature field plants or in seedlings that are grown at 95–110°F. very tiny late stripes appear in the leaves (Figure 6C). Thus under certain conditions, perhaps when *En* is affected, mutation can occur.

The pg *locus—a hypothesis*

It has been demonstrated that the mutation event, *pg* to *Pg*, is activated by a factor, Enhancer (*En*), that may be located at the *pg* locus or elsewhere in the chromosomal complement. In the presence of *En* mutable seedlings arise while in its absence the *pg* allele is stable. It has also been demonstrated that independent *En* arises by transposition from the *pg* locus. In addition, *En* could be present on more than one non-homologous chromosome.

It is assumed that the pale green mutant allele is an inhibited dominant green and that the mutation process is an uncovering-type event in which the inhibiting factor, *I*, is removed from its location at the *pg* locus. The removal of the inhibitor, which results in green tissue, is controlled by the Enhancer.

On the basis of these considerations the *pg* locus may be pictured as in Table 13.

There is some suggestion that *I* and *En* are more closely related than previously supposed. This is inferred from the observation of individual stripes and sectors of mutability in pg^s plants. These mutable sectors in stable seedlings (Figures 2A and 3) indicate that mutability has been induced at the otherwise stable locus. Such a possibility is strengthened by the isolation in stable stocks of a newly arisen mutable allele. In tests, this new mutable was found to be of the autonomous type. It follows, therefore, that in this case, *En* arose at the locus. Perhaps the *En* factor arises from the *I* factor that is associated with the pg^s allele.

New m type F_2 progenies have also originated in outcrosses of pg^m stocks containing independent *En*. Four m type F_2 progenies were observed in such outcrosses (Table 2) and two were used in further tests (Table 12). It is proposed that *En* arose from *I* material at the *pg* locus.

Other evidence supporting the relationship between *I* and *En* is seen in the

TABLE 12

Summary of F_2 progenies resulting from the outcross to various inbred lines of two m type progenies derived from the outcross of independent En

1957 source of pg^m	*pg* segregation of 1957 plant *Pg*	*pg*	pg^s	pg^m	1958 family	Classes of F_2 progenies G	s	m and s	m	Total
530–4	148 :	30	4	26	1285	17	2	3	5	10
530–5	118 :	27	3	24	1286	11	5	4	5	14

TABLE 13

The characteristics and hypothetical designation of each of the phenotypes associated with the pg *locus*

Seedling phenotypes	Genetic symbol	Hypothetical designation of the alleles	Nature of mutability
pale green stable	pg^s	*Pgl*	few *Pgl* → *Pg* somatic changes; *Pgl* → *PglEn* 1/512
pale green mutable	pg^m	$\widehat{PglEn}$	Enhancer at the *pg* locus. Many $\widehat{PglEn}$ → *Pg* somatic changes, $\widehat{PglEn}$ → *Pgl* and *Pgl* + *En* in more than 35% of gametes
pale green mutable	pg^m	*Pgl* + *En*	Enhancer at an independent location Many *Pgl* to *Pg* changes under influence of *En; Pgl* to $\widehat{PglEn}$ under influence of *En*
normal green	*Pg*	*Pg*	
pale green mottled	pg^{mo}	*Pgl'*	Mutates to *Pg* autonomously

interaction effect of pg^s and *En* upon pg^{mo}. In the hybrid, pg^{mo}/pg^s, the seedlings are stable. The characteristic pg^{mo} expression is inhibited. Likewise, the independent Enhancer causes pg^{mo} to appear stable. Thus the *I* element at the pg^s locus and independent *En* act in a similar manner upon the pg^{mo} allele.

I and *En* therefore appear to be related although they differ in activity. *I* inhibits the expression of the normal allele (*Pg*) whereas *En* inactivates or causes the removal of *I*. Whether the difference in activity of the two elements is a question of position or chemical composition can only be conjectured.

The inhibitor of the pg^{mo} allele is designated *I'* and the reason is as follows: the pattern of the pg^{mo} allele (Figures 6 and 7), is suggestive of only partial inhibition of the normal green allele. The inhibiting effect of *I'* seems to be weaker than the effect of *I*. *I* and *I'* may differ only in the strength of their effect on *Pg*.

GENERAL DISCUSSION

Many properties not previously associated with genes have been found in analyzing mutable gene systems in corn. These include the transposition of gene elements from one position to another in the genome, the mutation event at a locus due to the removal of the inhibitor of dominant gene expression, the modification of gene activity, the control of the rates of the reversion process and the induction of mutability at previously stable loci.

Evidence for the transposition of factors, a feature not commonly exhibited by genetic units, comes from linkage studies (McCLINTOCK 1956) as well as from the observation in the *pg-En* and other systems that elements are relocated (a shift from autonomous to independent control of mutability). In addition, the different phenotypes of mutable pericarp represent another instance of a transposition event (BRINK and NILAN 1952; BRINK 1954).

McCLINTOCK (1956) and BRINK and NILAN (1952) and BRINK (1954) have presented critical evidence for the presence in mutable gene systems of particulate inhibitors that cause inhibition of the normal functioning of the gene. Twin sectors of altered phenotypes are interpreted as the result of simultaneous events involving both the loss of a factor (the inhibitor), and its transposition to a new location. These correlated events are instances of undeniable proof that mutation is an uncovering-type action and they attest to the concept of transposition of factors. The nature of the origin of twin sectors obviates any possibility that elements already present are activated.

Additional evidence for the inhibition phenomena comes from a study of the origin of new mutables. In most cases these new mutables arise from previous dominants. New mutables have arisen in *Ac* stocks and respond to *Ac* control (McCLINTOCK 1953).

One new mutable discovered in an *En* stock (PETERSON 1953, 1957), a_1^m, has now been found to be controlled by *En*, but it does not respond to *Ac* or *Dt*. This type of evidence shows that the transposable parts of a mutable system can become associated with other loci and, in addition, that the specificity of such units indicates an origin from an existing mutable system.

The mechanism of transposition is not readily analyzable. It is clear, however, that it is not associated with crossing over events since the mutation occurs in somatic tissue as well as in plants that are hypoploid (derived from crosses with B-translocation stocks—ROMAN 1947) for the chromosome bearing the mutant gene.

Among the various problems associated with mutable genes in corn, two are relevant to the following discussion and they deal with the two units of the mutable system. First, there is the problem of the control of gene action by an accessory element, an inhibitor, adjacent to the locus, and the second problem involves the mechanism of removal of this inhibitor.

With reference to the nature of the control of gene action, McCLINTOCK (1951) favors the view that the presence of heterochromatic elements interferes with the ordinary functioning of a gene without changing the gene itself. BRINK (1958) has recently advanced a modification of this concept in which he suggests that accessory elements interfere with the structural reproduction of the normal genetic units. The subsequent disarrangement of these genetic units would result in their malfunctioning. The proposal by SMITH and SAND (1957) that mutability at the *v* locus in Nicotiana is associated with cell heterochromatization coincides with the above mutable gene hypotheses. In the *Spm* system (McCLINTOCK 1956), inhibition of genic functioning is controlled from an independent position and is not caused as in other systems by the proximity of the inhibitor to the locus. Most evidence obtained from phenotypic reversions with mutable genes supports the view that there is an interference in gene function while the gene itself remains unaltered. This is obvious from the fact that removal of the accessory element results in the immediate normal expression of the

gene. The origin of new mutables suggests that the inhibiting factor is nonspecific and can interfere with the functioning of a great many loci.

In any interpretation of the characteristics of different mutable genes, consideration must be given to the origin and specificity of two–part mutable systems (such as a_1-*Dt*, a_1-*Spm*, *Ac-Ds*, *En-I*). The nature of the specificity is evident in a review of the numerous systems associated with the a_1 locus—RHOADES (1941); MCCLINTOCK (1951, 1956); NUFFER (1955); RICHARDSON (1956); PETERSON (1957). It is significant that although the locus and the phenotype are identical, removal of the specific inhibitor (*I* or *Ds*) is accomplished only by its specific controller (*En* or *Ac* respectively). Furthermore, in the case of the pale green mutable system *En* appears to arise in the presence of *I* and this may be a clue to the individual specificity of systems. Both the specificity of control and the origin of the second member of the system are reminiscent of antibody-antigen relationships.

The mechanism of removal of the inhibitor (*I* or *Ds*) by the activating element (*En*, *Ac*, *Spm* or *Dt*) is a subject of speculation. It appears to be influenced by changes in temperature, by the presence of B chromosomes and by the age of the tissue. The possibility that these elements (*En*, *Ac*, *Dt*) exert a chemical control on the cell environment (RHOADES 1941) is strengthened by two different types of experiments: (1) STADLER's (1944) failure to remove the inhibition with X-rays and (2) BRINK's (1959) recent demonstration that a physical association is not necessary to effect the mutation event.

In conclusion, it can be stated that mutation in the mutable gene systems studied involves the operation of two related units within the cell environment. The mechanism of their operation as well as the modifications they invoke within the cell remain speculative.

SUMMARY

A mutable pale green gene that appeared among the progeny of maize kernels exposed to irradiation at the Bikini tests is herein analyzed.

Two distinct seedling phenotypes, pg^m and pg^s, occur in addition to the normal green plants.

pg^m is the mutable form and is controlled by a factor, Enhancer (*En*), which may be located at the locus (autonomous control) or apart from the locus (independent control).

pg^s is the stable form and lacks *En*.

It is hypothesized that there are two components in the pale green mutable system: *I* (Inhibitor of genic activity) located at the *pg* locus, and *En*, which can be located in various positions, and which causes the removal of *I*.

The pattern of mutability closely parallels MCCLINTOCK's mutable gene scheme; inhibition of normal genic elements in no way structurally alters the elements themselves.

Numerous experiments which illustrate the relationship between *I* and *En* are

discussed as well as the transposition of these components and the induction of mutability at previously stable loci.

A new mutable, pg^{mo}, and its relationship to *En* is also analyzed.

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Bristol Laboratories, Inc.
P. O. Box 657
Syracuse 1, New York—1957

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

Colonial Poultry Farms, Inc.
Pleasant Hill, Missouri—1958

DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Ghostley's Poultry Farm
Anoka, Minnesota—1956

Greenwood Seed Company
Greenwood Farms
Thomasville, Georgia—1959

Kimber Farms, Inc.
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nicholas Turkey Breeding Farms
Sonoma, California—1958

Nichols Poultry Farm
Kingston, N. H.—1950

Northrup King & Co.
Minneapolis 15, Minn.—1957

Pfister Associated Growers, Inc.
Aurora, Ill.—1957

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

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